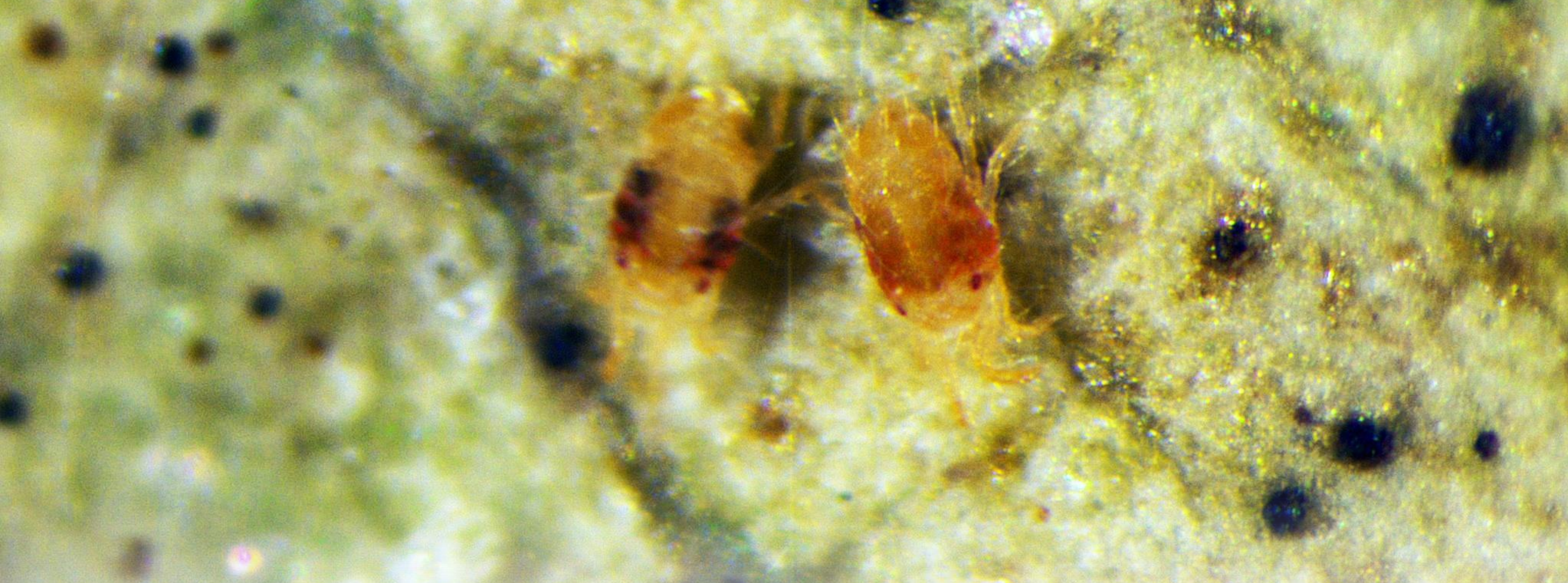

Comparison between different Tetranychus urticae control methods on Greenhouse Roses:

Solving the FLORALVES Case-study

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

Dissertação de candidatura ao grau de Mestre em Engenharia Agronómica submetida à Faculdade de Ciências da Universidade do Porto.
O presente trabalho foi desenvolvido na empresa FlorAlves, sob a orientação científica da Professora Doutora Ana Álvares Ribeiro Marques de Aguiar e coorientação da Professora Doutora Susana Maria Pinto de Carvalho

Dissertation for applying to a Master's degree in Agricultural Engineering submitted to the Faculty of Science of the University of Porto.
The present work was developed at FlorAlves Company, under the scientific supervision of Ana Álvares Ribeiro Marques de Aguiar, PhD and co-supervision of Susana Maria Pinto de Carvalho, PhD.

À minha família, à minha cara metade e ao futuro

"Why do we fall? So we can learn to pick ourselves up!"

- Alfred

Agradecimentos

A todos, não a alguns, nem tão pouco a nenhuns, mas a todos! A todos os que de alguma forma, em algum momento, vago, especifico, ou momento algum me acompanharam, de perto, de longe, mas que sobretudo marcaram e que fizeram toda uma roda caminhar sobre o caminho que aqui encerro, agradeço: Um eterno e enorme Obrigado!

À minha orientadora **Professora Doutora Ana Aguiar**, por me ter dado as linhas orientadoras e a 'liberdade' para poder crescer, aprender e apreender, podendo assim entender e compreender a minha tarefa pela exigência do empenho e de tomada de decisão. Os seus ensinamentos, dicas, criticas, guias, foram fulcrais para poder chegar a este ponto com o conhecimento que adquiri. Obrigado!

À minha coorientadora, **Professora Doutora Susana Carvalho**, pelo acompanhamento e pelo incentivo, por ter me colocado a pensar mais 'além' e a construir soluções para problemas que pareciam insolvíveis. Pelo seu precioso tempo, em que em poucas palavras muito disse para me manter a caminho. Obrigado!

Ao **Engenheiro Sérgio Alves**, 'chefe' da Floralves, por ter tido sempre as portas abertas e uma disponibilidade engenhosa de trabalhar e guiar! Um exemplo do que qualquer estudante aspira ser no futuro: sucesso, trabalho e pés bem assentes… no futuro! Sempre disposto a criar e a construir, e como um verdadeiro Humano, fazer crescer do pedacinho, um grande caminho! Obrigado! Estendo este agradecimento a todos os colaboradores da Floralves!

Palavra de apreço à minha colega **Susana Machado**, pela sua presença e ajuda, espero que o trabalho que desenvolvemos juntos te tenha enriquecido e saúdo-te igualmente no final desta tua etapa.

Um enorme obrigado a esta casa – à **Faculdade de Ciências da Universidade do Porto** – e todos os docentes deste meu percurso, do departamento de Biologia ao departamento de Geociências, tão longo ele é pela Nacional 13 até Vairão. Todos eles contribuíram para cada passo dado e cada passo que me falta tomar! Uma nota de apreço aos docentes e colegas da **Faculdade de Engenharia da Universidade do Porto** pelo acolhimento e aulas de grande fervor, porque "a prática aperfeiçoa".

A todos os colegas e amigos que nasceram deste Caminho iniciado em **2013, os Agrónomos** que em tantas viagens me acompanharam, em tantas conversas nos perdemos, mas que algo em comum encontramos! Á 'procissão do girassol', pois, como bem sabemos, o que tem ligações à terra, tem por necessidade banhos de sol! Obrigado 'jovens'!

A todos os **'bichinhos'** que me deram toda a força a ter, desde o primeiro dia nesta instituição, vontade de continuar o caminho e não desistir! A TODOS sem exceção, presentes de 2008 a 2014, fizeram parte de grandes momentos, de bons e maus, mas daqueles que marcaram para a vida e que ficam para sempre! Um grande Obrigado e "continuem com essa alegria de viver"!

À **GRANDE MALTA DO COSTUME**, agradeço vos por tudo e mais alguma coisa, são vocês os amigos que estão realmente lá! Não interessa onde, nem quando, interessa o 'lá', o 'cá', o SEMPRE. Por todos os momentos que passamos, e que com a maior certeza do mundo passaremos, dos nossos jantares, às nossas desvairadas peregrinações 'Maienses' (destes e dos próximos anos, porque não há cá velhos!). Por tudo o que contribuíram para quem eu sou hoje, e quem serei amanha, por serem aquela velha bengala que sempre nos apoia e que um dia por certo virará andarilho! Lembrem-se, estejam onde estiverem, temos carta registada para marcação mensal – ainda que por certo teremos de juntar uns quantos eventos de uma vez só! A minha eterna e profunda gratidão!

À **Ana**, que o agradecimento que deixo aqui se ecoe por uma e outra vida! O Amor não é eterno, é efémero, por isso tal eco, contigo sei que este foi o primeiro tijolo de uma grande casa! Agradeço por tudo o que passamos, e por tudo o que me fizeste ofereceste e fizeste crescer! De todas as palavras, incentivos, e força, por não me largares e não me deixares desistir! O ânimo, os conselhos, os abanões! Juntos nada é impossível, não é difícil! É o que é, uma estrada nova neste caminho sinuoso que é a vida!

À **minha família**! Aos meus **pais**, ao meu **irmão**, à minha **tia**, à minha **avó** e ao meu **avô**! Obrigado a todos vós! Vocês deram me os pilares de uma grande fundação para poder construir todo o meu presente, todo o meu futuro!

Aos meus **pais** por todo o esforço em oferecerem me aquilo que eles não poderão ter, a luta foi e é difícil, mas as vitórias só se alcançam indo à guerra! E eu sei que vocês batalharam com tudo nesta luta que é a vida! Obrigado!

Ao meu **irmão**, por ser uma imagem de mim, de poder olhar para ele e ver de onde ‘tudo’ começou! Agarra a vida com garras e dentes, pois se tu não o fizeres, ninguém o pode fazer por ti! Tens muito para caminhar, mas não te falta gente para te puxar! Obrigado!

À minha **tia**, **avó** e **avô**, porque cresci com eles, e o seu acompanhamento é de uma escola totalmente diferente, pregada pelos ensinamentos da experiencia e os problemas da vida, nenhum manual ensina isso, nenhuma calculadora resolve tais! Obrigado!

Este foi o ano de mudança, aquele que marcou um fim, um fim que não é um fim, é apenas um novo início! E este é o primeiro passo nessa direção.

Resumo

As rosas são uma das mais importantes culturas na floricultura Europeia e Portuguesa, sendo a sua aparência o seu principal fator de avaliação e valorização. A FLORALVES, uma empresa de produção de rosa em estufa da região norte de Portugal, Vila do Conde, foi utilizada para o estudo devido à presença de longo prazo do Aranhiço-Vermelho, *Tetranychus urticae (Acari: Tetranychidae)*.

T. urticae, um acaro fitófago, provoca danos nas plantas devido aos seus padrões alimentares, sugando o conteúdo das células, cicatrizando e reduzindo as capacidades de fotossíntese da planta, deixando-a num estado de produção e de qualidade débil, fator critico para o mercado de flores de corte. Tais perdas têm sido recorrentes na estufa nos últimos anos apesar dos esforços para corrigir a situação. Apesar de estudos precedentes recorrendo a um vasto leque de produtos e técnicas terem sido realizados, não foram obtidos resultados positivos nem foi encontrada qualquer solução válida para fazer face ao problema.

De forma a delinear o desenho experimental adequado na procura de uma solução para este problema, foi conduzida uma avaliação preliminar, recorrendo a uma revisão bibliográfica. Dado o mecanismo altamente adaptável de resistência do *T. urticae*, foi colocada a hipótese de que os métodos de controlo convencionais acabavam por ver a sua eficiência degradada ao longo do tempo. A resistência ao tratamento era potenciada pelas repetidas e elevadas doses dos químicos utilizados. Assim, o uso de agentes auxiliares (como os ácaros como predadores auxiliares o são) foram recomendados ao produtor, no sentido elevadas taxas de sucesso observadas em casos de estudo semelhantes.

Um estudo comparativo de três diferentes métodos de controlo foi efetuado: 1. Uso de um bio pesticida de síntese, com um princípio ativo convencional, Abamectina (**Sapec BOREAL**); 2. Uso de um bio pesticida de síntese recentemente desenvolvido com uma nova formulação (**Cultaza SERV-MITE**); 3. Uso de **ácaros auxiliares** (*Neoseiulus californicus* e *Phytoseiulus persimilis*, fornecidos pela Koppert). Por razões logísticas, como a divisão e posicionamento das naves da estufa, e por recomendação do produtor, a variedade estudada foi a “White Naomi”, presente nas naves a noroeste da estufa.

De forma a gerir a evolução da praga, foi necessário um método de estimativa de risco adequado e simples para usar no terreno. Com material e dados preliminares recolhidos na estufa, e observados em laboratório, uma escala de intensidade de ataque de 4 níveis por contagem de indivíduos foi elaborada. Durante a observação bissemanal na estufa, as formas adultas e ovos de T. urticae presentes quer no pulmão, quer na zona de produção, foram avaliadas através desta escala. Estes dados foram usados quer para a comparação final dos diferentes tratamentos, como para tomadas de decisão – desde a aplicação de tratamentos adicionais à gestão da cultura, tendo os dados recolhidos sido partilhados com a Koppert para tomadas de decisão nas largadas de **auxiliares** adicionais. O acompanhamento foi realizado num total de 15 semanas, dividido em dois períodos, antes e após a décima semana, momento em que foi necessário proceder a uma poda conducente a uma redução drástica da população de ácaros.

Durante o primeiro período todos os tratamentos obtiveram resultados semelhantes, com grande intensidade de ataque e ocupação foliar, resultante da drástica proliferação da praga, culminando numa planta de fraca qualidade. Ao final da décima semana, o produtor tomou a decisão de remover todas as hastes irrecuperáveis, reconstruindo o pulmão – reduzindo para valores aceitáveis o nível de ataque. Por razões logísticas e económicas, o tratamento com **SERV-MITE**, foi descartado em detrimento do **BOREAL**.

Os resultados obtidos nas semanas posteriores revelaram que as plantas tratadas por este, obtiveram uma pequena, mas reduzida, melhoria. As qualidades mínimas desejadas não foram atingidas, dada a alta intensidade e ocupação de ataque. Em contraste, as plantas que receberam os **auxiliares**, obtiveram uma melhoria significativa, reduzindo os níveis de ataque a um nível quase residual e animador.

A qualidade e a produtividade foram avaliadas à colheita através de medição da altura das hastes, largura do botão floral e número de hastes comerciáveis. Como esperado das observações em campo, a colheita obtida das plantas tratadas com **auxiliares**, superaram os requisitos mínimos de qualidade e dobraram a produção das obtidas do tratamento com **BOREAL**.

Assim, os nossos resultados contribuíram para a resolução do problema inicial estando agora a ser implementados no tratamento da praga da cultura. De facto, o produtor converteu toda a sua estufa ao uso de auxiliares, reduzindo a necessidade de uso de pesticidas, e diminuindo a praga para valores residuais. Apesar destes resultados positivos e promissores, um novo acompanhamento deverá ser realizado de forma a determinar os benefícios deste tratamento a longo-prazo.

Palavras-chave: *Tetranychus urticae*; Estufa; Rosas; White Naomi; Estimativa de risco

Abstract

Valued for appearance, roses are one of the mostimportant crop in European and Portuguese floriculture market. A greenhouse rose production case-study, FLORALVES, on the northern region of Portugal, Vila do Conde, exhibited a long term problem with two-spotted mite infection, *Tetranychus urticae (Acari: Tetranychidae)*.

T. urticae inflicts damage to plants due to its pierce and sucking feeding patterns, scaring and reducing plant photosynthesis capabilities, leading to severe production and quality losses, critical to the cut flower's market. Despite all efforts to solve the situation, with previous studies recurring to a variety of products and techniques, no positive results were met to this production.

A pilot review was done to sketch an appropriate experimental design underlined the highly adaptable resistance mechanism of the *T. urticae*, hypothesising standard control methods lose their efficacy over time - worsen by the high chemicals dosages employed in the field. The use of a Biological Control Agent (BCA) (such as auxiliary predatory mites) shown high success rates in similar case-studies and was presented to the farmer.

A follow-up and comparative study was done using three different control methods: 1. Standard bio pesticide product, using the active compound Abamectine (**Sapec BOREAL**); 2. Newly developed bio pesticide, using a new formulation (**Cultaza SERV-MITE**); 3. **BCA**, auxiliary predatory mites (*Neoseiulus californicus and Phytoseiulus persimilis* provided by **Koppert**). For logistic reasons, such as greenhouse modules division and positioning, and with the farmer recommendation, the "**White Naomi**" variety present in the north-western modules was the selected for our study.

In order to easily manage the pest evolution data a suitable, and user-friendly, risk assessment method was developed. Using preliminary data, gathered from in-lab observation of roses leaves gathered at the greenhouse, we developed a 4-level Attack Intensity Scale. This scale was then used during the bi-weekly on-site observations, to score the *T. urticae* adult forms and eggs present on rose leaflets, both in the maintenance layer (the "lung") and in the production layer. This data was then used for end results comparison and decision making, such as, pesticide application, additional treatments and crop management procedures. Furthermore, results were shared with Koppert for auxiliary mites release patterns decisions.

The follow-up study lasted a total of 15 weeks and was done in two periods: until and after the 10th week. This division was due to the need of a pruning and a treatment policy reform. During the 1st period, treatments had similar poor performances, with high attack intensity levels and leaflet occupation, result of a drastic pest proliferation, what culminated in a degraded plant quality. In fact, at the end of the first 10 week period the farmer had the necessity to remove all the major attacked stems, rebuilding the plant maintenance layer. Due to logistic and economic reasons, **SERV-MITE** was ruled out in detriment of **BOREAL**.

In the following 5 weeks, the plants treated with **BOREAL** presented a small but negligible improvement, as it did not meet the minimum expectations, maintaining high attack intensity and occupation levels. On the other hand, plants treated with **BCA** displayed a significant improvement, reducing mite attack levels to a bare minimum.

Quality and productivity were measured at harvest using stem weight, flower bud width and number of saleable flowers. Moreover, as expected from our field evaluation, plants harvested after **BCA** treatment meted and surpassed the minimum requirements in plant quality parameters, with production values two times higher than those obtained from plants treated with **BOREAL**.

Our results contributed to solve the problem that had been recurring for the past years, and can now be used for future productions. In fact, following the end of the essay, the farmer converted the whole greenhouse to the use of **BCA**, reducing the use of pesticides and the number of *T. urticae* infection to a bare minimum. These positive and promising results must be further validated by follow up studies that assess the overall **BCA** benefits and evaluate success of the treatment in long-term.

Keywords: *Tetranychus urticae*; Greenhouse; Roses; White Naomi; Risk Assessment

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€	Euros
ABA	Abscisic acid
BCA	Biological control Agent
EC	Electric conductivity
ET	Economical Threshold/Injury Level
FBW	Flower Bud Width
ha	Hectare
IPM	Integrated Pest Management
ML	Maintenance layer
NBP	New Biopesticide
NL	Netherlands
ºC	Degrees Celsius
PHS	Post-Harvest Solution
PL	Production layer
SBP	Standard Biopesticide
SH	Stem Height
TPL	Taylors Power Law
UK	United Kingdom

I. Introduction

1. Background

Portuguese floriculture market generates near 490 Million euros with Cut Flowers [1,2] representing three quarters of Portuguese Floriculture Holdings in which Roses occupy a third, thus being one of the most important crops in the Portuguese floriculture market [3]. Floriculture market is based upon fresh and healthy looking cut flowers sold in a bunch, bouquets or individually, depending on the choice of market channel and final costumer. As such the product appearance is of the highest importance and a defining point for market value and successful sales, granting the desirable income to the farmer [2].

Roses fit in this category, and market demands good quality and good looking roses, as such any disease and pest are of the upmost importance for the end-value of the product and a defining problem for the farmer. Greenhouse Roses are typically grown in beddings in an hydroponic system, with controlled parameters in order for an optimum growth and quality, but this closed systems can lead to problems with pests that thrive in hot-humid climate offered by this type of housing [4–6].

Tetranychus urticae is a prime example of this problem; a polyphagous mite, attacking the host plant sucking the leaves cell contents, scaring the tissue and reducing the photosynthesis capability of the plant, leaving it weaken and greatly reducing its growth capability [7–9]. Although its control was maintained with an array of products, the pest adapting mechanism as lead to major resistances to almost every chemical solution leaving the farmers with less resources to fight the *T. urticae* attacks [10–12].

With this many farmers are turning their attention to the use of beneficial predatory mites as *Neoseiulus californicus* and *Phytoseiulus persimilis* [8,13–18]

In the present study we did a follow up on a greenhouse rose production, FLORALVES, situated in Fajozes, Portugal, which *T. urticae* presence was a major problem, with yield and quality losses, along many failed attempts with different crop protection methods with different chemicals and strategies.

Preliminary bibliographic research and attending the past control methods, a study was proposed on a premise of a possible biologic unbalance – either from very likely grown chemical resistant pest, but the need of a biological control agent, like a predatory enemy [19,20].

The present essay compares different control methods in one of the varieties present in the greenhouse – *Rosa spp* Var. White Naomi – introducing predatory mites as one of the methods along with a control treatment (the current main pesticide) and an alternative new bio pesticide.

Although being a common and uprising pest, scientific data on *T. urticae* was scattered among different crops, standalone essays, and in lab chemical resistance testing. A lack of an easy and user-friendly risk assessment method and a practical in field evaluation was found and needed to be addressed [13].

In order to culminate this in depth Bibliographic Review was done and a new risk assessment was proposed from at-field experience and in-lab visualization.

2. Aims

The aim of the study was to follow the pest evolution with different control methods, evaluating which one performs best reducing *T.urticae* population.

In order to balance field sampling and decision making, a new, practical and user friendly, non-invasive, risk assessment method was developed and proposed in order to optimize data sampling and decision making.

Quality and productivity data was also evaluated for further comparison.

Along with essays aims, the practical aim to find a conclusive solution to the farmer problem was a top priority and the main purpose of this essay.

I. Bibliographic Review

1. Roses

Roses (Rosa spp.) are woody perennial (Genus Rosa, Rosaceae family), varying in size and colour depending on the species. Genus Rosa is complex and complicated mainly due to the vast amount of publication names, with 100 to 250 wild species. Hybrids development occurred by crossbreeding during the last centuries which made near to impossible to distinguish a wild species from a hybrid [21].

Roses can be di-, tetra-, penta- or hexaploid, with respectively 14, 28, 35 or 42 chromosomes, and this with small cross boundaries between the species explains the breeding potential of these plants [21.22].

1.1. Roses throughout the ages

Most species are native to Asia, but there are some native to Europe, North America and Africa. Many of the first species were found in Europe, but nowadays Garden roses are normally complex hybrids result from centuries of genetic selection in China, encouraged by the trade routes through the famous Silk Road. [22]

Among ancestors are species like *Rosa gigantea*, which was then brought back to Europe and hybrids were developed in Lisbon circa-1896; and *Rosa chinensis*, which had a variety of colours from red, pink, yellow and white, thought to be the main common ancestor of many actual hybrids that were naturally occurring along the centuries [21.22].

These roses where of the upmost importance, as in contrast of their European counterpart, that only bloom once a year, these could bloom in spring, summer and fall. [23]

Hybrid gardens in the late XVII century held responsibilities to new hybrids and new genetic changes to the original ‘tea flowers’ route. Species like “Slater’s Crimson”, “Parson’s Pink”, “Hume’s Blush” and “Park’s Yellow” were first identified from these gardens, but soon connections were made to early paintings and known to also had origins in Europe. Many hybrids were then born from the “rose breeding era” in Europe, where new tea scents were search from hybridising the roses.[22,23]

Technologies evolved and where many roses had trouble developing ‘hot-houses’ were installed and there were rose breeding centres in cold weather places like London or Berlin. In UK, the national rose society was born, bringing to light newer technologies of rose breeding and growing, and setting new standards for rose quality, creating a live and growing market [22].

In the beginning of the XXth Century, roses, that where until then grown for Tea market demand, were starting to be seen for decorative purposes, with rose flowers being used in ever growing situations like flower arrangements and table decorations; and in time found their way into the fashion business in clothing and hair adornments.

The ever so demanding market for roses made use of the train network, and circa 1937 the use of refrigerated containers were used so roses could go to the farthest markets like the Scandinavia [21.22].

In time, and due to the First World War, Tea Roses were decaying in popularity and over 500 different varieties of roses were drop from growing catalogues.

In time, the market bloom for ornamental and cut roses, where the importance of constant flowering plants is the core part of the business. [22–24]

Roses reach commercial maturity at bud are then harvested and stored under refrigerated conditions until reach their sale point. Usually are sold as bunches of 20 unities or used in bouquets or flower arrangements. [2,25]

1.2. Agronomic data

1.2.1. Botanic proprieties

Woody shrubs shooting stems with composite leaves – leaflets – occurring spirally with flowers bud on the tip, their horticultural classifications vary. Based on the number of flowers in the inflorescence, the size of the flower, shoot length and plant shape, it is divided in different groups [22,24]:

- Tea- hybrids, with one or more flowers per stem
- Polyantha, with clusters of many small flowers
- Polyantha hybrids or Floribunda and Grandiflora, with a number of flowers in between those of the previous two groups

Varieties are then catalogued by colour, shape of the flower base, shape and position of the sepals and petals, shape of the bud and the open flower.

Commercial rose's varieties are classified by bud size and their number in the inflorescence [2,24]:

1.2.2. Climate

Wild roses are found in the northern temperate climate zones and subtropics.

Greenhouse allows rose cultivation in virtually everywhere in the world, but its limited by the type of greenhouse, the equipment used, which differs from the specific climate of where the greenhouse is, and the variety used. Morphological aspects of the same variety can even be completely different on these climate factors.

For instance number of petals and colour intensity increases with lower temperatures but also a decreased length-width ratio which can lead to misshaped flowers [6,24].

1.2.3. Shoot formation

A new shoot can develop from a bud in the leaf axis ('bud break'), normally due to the cutting of an old shoot, ceasing the apical dominance supressing bud growth. Time from bud break to blooming can vary with different varieties and temperatures, taking longer on colder climates, although stem length is increased [24].

1.2.4. Hormonal regulation

There are 4 known regulators that influence rose growth [24]:

1. **Auxins**, promoting formation of adventitious roots, applied for propagation aiding, it can have an antagonist effect in higher concentration, causing root growth inhibition.
2. **Cytokinins** reduces the aging process, tuning growth control. Water deficit or shortage of nutrients and oxygen can reduce ctyokinins and inhibit aerial development.
3. **Abscisic acid (ABA)** promotes dormancy and inhibits growth. Stimulates senescence in stressful situations.
4. **Ethylene** has almost the same effect as ABA. It is of upmost importance in post-harvest quality as it promotes flower wilting. During crop growth its presence should be monitored as can easily originates from bad combustion of fuel by farm vehicles or greenhouse heating systems.

1.3. Greenhouse roses production

1.3.1. Greenhouses 101

Greenhouse fundamentals are to offer the best climate for a crop and giving control over its management. Weather can be maintained over control, avoiding harsh crop conditions, and a wide array of greenhouse equipment can give us as much control over the establish greenhouse climate as we want, depending on the available systems.

Climate, water and nutrient can be programmed for automatic management, offering fine-tuned optimum response to crop needs [26].

1.3.2. Technology overview

On table 1 it is possible to overview standard differences between 3 different technology graded greenhouse: from a basic, standard-tech greenhouse to a full-fledged highly capable, typical NL greenhouse.

Table 1. Greenhouse technology range overview. Adapted from [24]

Element	Standard-tech	Above standard	High-Tech (Netherlands)
Light	Plastic greenhouse cover; shading nets	Plastic greenhouse cover with diffuse proprieties; shading nets	Glass, standard or with antireflection and diffusing proprieties; assimilation lighting
CO2	None or fixed window opening, without CO2 inlet	Flexible window openings to maximize Co2 inlet if necessary; mechanical ventilators inside the greenhouse	Industrial CO2
Temperature	None or fixed window opening; passive, natural ventilation	Flexible window opening; passive, natural ventilation	Flexible window opening; active cooling or heating.
Air humidity	Fertigation on fixed hours, leading to water shortage during mid-day	Automated increase of fertigation frequency during mid-day, maintaining high transpiration	Air treatment unit
Water	Fertigation on fixed hours, use of EC.	Fertigation on the basis of radiation sum; use of EC.	Water content sensors provide information on the solid/substrate status to the computer; shortages are replenished
Water source	Surface water	Surface, bore hole and rain water; basins; recirculation with disinfection	Surface, bore hole and rain water; large basins; water purification before using, recirculation with disinfection; minimal drain
Climate homogeneity	Only natural ventilation	Mechanical ventilators	Greenhouse dimensions specifically determined; top or bottom air supply; air treatment units
Nutrients	Fertigation on fixed hours; use of EC	Fertigation on the basis of radiation sum; use of EC.	Ion-specific sensors provide information on the soil/substrate status to the computer; shortages are replenished
Energy	Low requirement; obtained from the net	Higher requirement; obtained from the net	Highest requirement; obtained from the net and from solar panels; WKK installed

Automation	No automation or time-clock	Computer-based fertigation; some climate registration	Computer-based fertigation and climate management; climate and other sensors
Cost	Low	Medium	Very High

Greenhouses can be classified by shape, materials, construction, ventilation or utility types [27].

On installation location certain aspects such as the land surface, soil proprieties, altitude, road accessibility and power and water availability should be taken in account, but also the greenhouse construction in order to obtain the desired proprieties [24,27].

1.3.3. Choosing a rose variety

The choice of the varieties to grow will influence the complete farm's strategy. This choice should be made by a balance between growth and market potential, so there is room for optimal growth conditions, production yield and economic investment balance while meeting up the market demand. Table 2 summarizes criteria to take in account during this choosing.

Table 2 - Variety decision criteria. Table Adapted from [24]

Production criteria	**Market criteria**
Climate necessities	Compatible with current strategy
Productivity	Number of supplying growers
Pest and Disease resistance	Market demand fluctuations
Success/Failure rate statistics	Market channels
Post-Harvest Sensitiveness	Vase life and quality
	Competitiveness

1.3.4. Weather, inside-out

There is a variety of ways you can manage the outside climate conditions, and this data is important in order to adjust greenhouse conditions to optimize crop conditions. Variable listed in the table 3 needs to be addressed in order to better control greenhouse crop production [24,27].

Table 3. Climate conditions outside and inside the greenhouse. Adapted from [24,27]

Outdoor Weather stations	**Greenhouse climate**
Temperature (ºC)	Temperature (ºC)
Relative humidity (%)	Relative humidity (%)
Radiation (J/m2)	Vapour Pressure (VD)
Wind speed (m/s) and direction	
Absolute air pressure (mBar)	

Climate data is important from installation to crop management; it enables decision making and pest and disease prediction.

1.3.5. Growth conditions

Greenhouse roses produce all-year round with periodical flower shoot cuttings in a cycle of growth and flowering. The shoot is harvested upon reaching commercial maturity in a period from 5 to 8 weeks, after a new shoot develops from the uppermost axillary bud [21.24].

Fertilization

In order to establish a fertilization formula sampling to soil (soil based systems) or water (hydroponic systems) need to be taken for physio-chemical lab analysis.

Usually a Spurway method is used, which gives information on available nutrients, and a general soil characteristics test, offering soil classification, organic matter, clay content and Calcium and pH-KCL data [24].

On a hydroponic system the source water that will be used needs proper testing too in order to make correction. Parameters like nutrient composition, electric conductivity (EC) and pH level have to be measure [28].

Growing Media

Soil

Roses withstand different soil types, as long as the characteristics on table 4 are met

Table 4. Soil requirements for rose cultivation. Adapted from [24]

Soil Proprieties demanded for Rose cultivation	
Good, homogeneous, stable structure	Homogeneous soil profile
Air holding in wet conditions	Good drainage
Good permeability	Constant groundwater level

Hydroponic System

Greenhouse rose productions are typically grown either in an open, non-recirculating nutrient system or a closed recirculating nutrient system [24]. The first were the classic method used that had been gradually switched onto the more environmentally friendly method of a non-wasted irrigation method, decreasing pollution and contamination [28,29].

This, however can bring us some downsides: disease control and a renewed nutrient solution.

Conclusions vary in different studies comparing growth conditions [4,28], some having found no difference in rose production or quality, while others found roses less vigorous in closed systems, with worse quality over-time [30]. Reasons could be due to problems in the nutrient solution, as a simple change in pH can alter nutrient uptake of the plants, and lead to bad results. Other reasons could be due to filtration problems and unwanted materials circulating in the solution. [4,28]

A set of characteristics must be found in the substrate in use for a hydroponic system, as listed in table 5, assuring the best support and growth capabilities to the plant.

Table 5. Subtract proprieties. Adapted from [24].

Subtract basic proprieties
Plant support
Low bulk density
Pore spacing for best air and water distribution
Water holding capacity
Rehydration capabilities
Drainage capacity
Durability
Easy management, availability and low cost

Irrigation

Based on previous climate data, crop evaporation and soil water content and drainage, an irrigation scheme needs to be addressed with daily irrigation crop demand and the irrigation supply method, volume and schedule [29,31].

Nutrition

A typical annual Nitrogen demand is around 12,9 to 17,2 g N per plant, with a lower absorption rate during shoot elongation periods and higher absorption when elongation stops . This was proven to be due to competition within the plant natural system for nutrients and photo assimilates, explained by the assimilates sinking during growth periods, where the plant upon low supply of nutrients, slow down or ceases growth until the plant can gather the necessary N to repeat the cycle. [32]

Other nutrients uptake, such as Phosphorus, Calcium, Potassium and Magnesium mirror the changes in the N uptake. Some of these elements have synergisms between them while other are antagonists which can be observed in the figure 1.

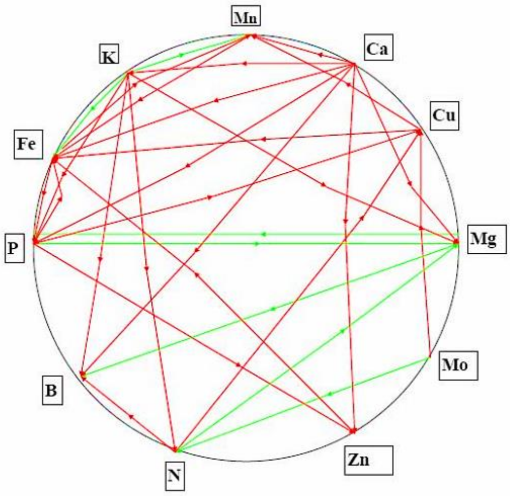


Figure 1. Synergism and antagonism between elements. Adapted from [24]. Synergisms are represented by green, while antagonism interaction is represented in red.

Fertigation

Technique of supplying nutrients to the crop by means of the irrigation system. In substrate systems, available nutrients are limited, and a frequent water and nutrient application is needed for good crop growth [24].

Since nutrient uptake can differ between elements, it is required not to eke out the absolute nutrient needs but to balance the application make use of ion-specific nutrient application. This is done by sample analysis of the plants or the fertigation media [24,29,30].

pH

pH is also an important factor as it control the amount of nutrient uptake since they differ at different pH levels; optimum pH level for roses is between 5,5 and 6,5 [figure 2] [24,29].

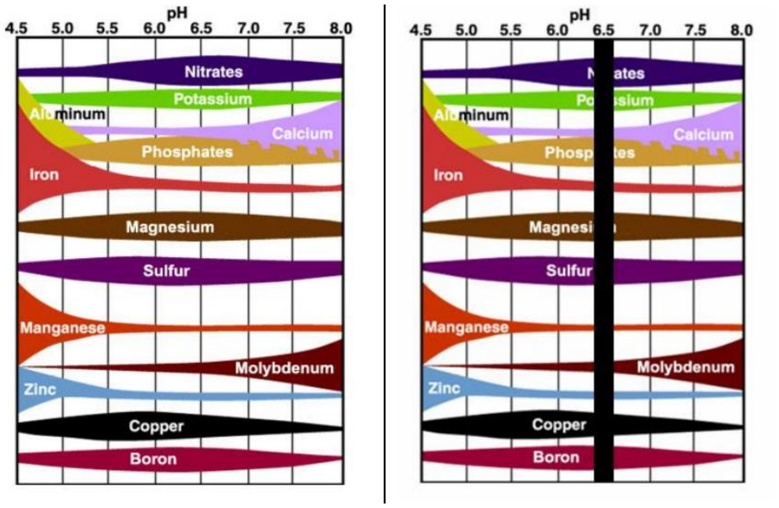


Figure 2- pH relation with nutrient uptake ability in Roses. Figure adapted from [24]

Electric conductivity

EC is a measure of the dissolved ions in the water. Simplifying, the lower the EC, the fewer nutrients contained in the water solution. EC level depends on the variety of rose used and cultivation system. An high EC lowers water uptake and leads to lower growth level, but also stronger stems and more colourful leaves – due to higher dry matter concentration; this is often a requirement in rainy seasons to reduce crop sensitiveness to diseases, as seen in table 6 [28].

Table 6. Optimal EC levels for roses grown in a Hydroponic system. Adapted from [24]

	Dry Season (µS/cm)	Rainy Season (µS/cm)
Drip water	1.4 – 1.6	1.6 – 1.9
Drain water	1.6 – 1.8	1.9 – 2.1

Fertigation systems

There are three types of applications techniques as seen in table 7:

Table 7. Fertilizer application method [24]

Method	Description
Continuous application	*Fertilizer applied at constant rate at all times, regardless of water discharge rate*
Three-stage application	*First stage of irrigation is only water; second stage begins after wetting, injecting the fertilizers; last stage used as a fertilizer flush from the system*
Proportional application	*Fertilizer injection is proportional to the water discharge rate*

Furthermore there are two different injection systems (table 8):

Table 8. Fertilizer injection and mixing method [24,29]

Injection type	Description
Inline system (direct injection)	*Use of separate tanks in the system: a number of fertilizing tanks (usually 2), and a pH control solution tank.* *Fertilizers are injected and mixed with irrigation water and, after a pH and EC reading, corrected with the solution in the pH control solution tank.*
Separated mixing	*Similar to the inline system, but mixing occurs before entering the irrigation water system for an accurate mixing*

1.4. Crop management

There are a number of practices in order to achieve a good, healthy crop maintenance and good end product [33]. Cultivated roses are a perennial woody shrub, forming constant new shoots, producing flowers, which are then harvested for commercial purposes [2].

The plant life span is about 4 to 7 years, as with age the flower quality decreases, which is then replaced with a new plant; this life span can be optimized with investment in the plant structure [24,34].

1.4.1. Installation and beginning

Roses plants are multiplied by vegetative propagation (either by cuttings, stentling or grafting) in nurseries where they are normally sold to end-producers with certification and quality control parameters [24,35].

Planting is often done before the rainy seasons, this can offer the intermediate conditions in terms of light and humidity so the plant can adapt itself [24].

Densities decision affects production rate and quality; lower densities gives good quality but low production, while higher densities maximizes production but the loss in quality in the following seasons is exacerbated [24].

Roses are planted in subtract or soil, which then the axillary bud will develop into a shoot [6]. This shoot will soon flower, but flower removal is needed so the remaining buds can snap out of dormancy, and them too can grow and form lateral shoots, repeating the process until a desirable number of shoots is achieved. During this time high Air humidity is needed to keep the crop moist and high radiation to favour crop growth [24,28].

Bending and Layer Structuring

About a month after planting primary shoot is bent, ensuring it to be as low as possible so it can induce new and strong shoots, where the production will be harvest from [24].

When the first shoots are ready for harvest, the first cut should be done above what will be the usual point of harvest, ensuring that two shoots can grow in the place of the one that has been harvested. Then all the thin and short stems should also be bent [24].

This will optimise all the rose structure, creating a **Maintenance layer** with high leaf area, commonly called "*Lung*". The top Layer will be the **Productive layer**, where all the grown stems will be harvested, as they will be the thickest and longest, following the typical plant apical dominance.

Crop Maintenance

In order to maintain good production and high quality stems, some steps need to be taken:

- The ***Maintenance layer*** will be the rose energy producer, made by the unsellable smaller stems, but if for some reason these are not available, a normal stem should be sacrificed in order to maintain full plant capabilities.
- ***Desuckering*** is a technique where small side branches are removed by hand as they don't offer any advantage and often use energy that should be redirected to the plant
- ***Pinching*** is a pruning method where the tip of a stem is removed in order to correct the remaining stems growth – for example when they are too thin. Pinching removes apical dominance, and energy and nutrients are redirected where they are needed. Pinching and the extent of its use depends on variety, growth and crop conditions.

1.5. Crop protection

Crop protection starts from the very beginning during the installation when choosing the production method, tools and specifications; it then continues while choosing the variety, as some have far better resistance to pests and/or diseases [24,33,36,37].

Pests must be kept at minimum level, below economical threshold (ET). This level is obtained by the interaction between Injuries – physical, crop, harm or destruction to a valued commodity by the presence of pests and/or diseases; and the Damage – economical value loss by result of Injury. For instance, plants often suffer some degree of injury that does not affect final yield or overall quality, and no treatment is economically justified by this. ET is the breakeven point between Injury=Damage, where actions must be taken for financial lost not occur. Usually there is a point before reaching ET where actions taken have better results, fewer losses, and pest/disease control is optimized so it will not break the ET barrier – this is called economic threshold [38,39]

Preventive and curative measures must be taken in order to sustain a wealthy and protected crop production [33,36]. Use of tolerant varieties, correct climate control in a greenhouse, insect nets and good overall sanitation measures are just examples of preventive measures. Curative measures have a higher cost footprint and some problems can even have no curative solution; these measures include phytochemicals applications or predator's releases for instance. Typically bellow Damage, or even Injury point, preventive measures are the ones to take on, while reaching ET, curative measures must take place [24].

Roses good disease management greatly depends on sanitation: old, and/or infected, crop removal and destruction, weed removal, good quality water.

1.5.1. Integrated Pest Management

Contrasting with old habits of 'use and abuse' of chemical overdosed applications, today farmers are growing towards sustainable agronomic practices, reducing pollution and toxicity footprint and working in an environmentally responsible matter using different methods to achieve better crop protection [20.33].

With this Integrated Pest Management (IPM) growth into a standard in current rose productions, reaching for biological equilibrium, looking into less chemicals and resourcing their methods into biological control agents (BCA).

Advantages goes beyond cost-wise applications, but in reduced toxicity and resistance build-up and often use as a sign of quality for the end product [10.24].

1.5.2. Application

Sampling and Risk Assessment

Sampling for pests and diseases should be done regularly in order to take measures before extensive damage can occur. A problems cost is as reduced as soon it is discovery, so spotting a problem early is a great investment.

Risk Assessment can vary on methodology, but typically a random but representative sampling is done throughout the whole greenhouse in order to have the best overview of the complete scenario [13].

Additional sampling can also be done with use of sticky cards above canopy level, as it attracts several insects and we can latter evaluate it. This is mainly used for trips and white fly [20.24].

Product applications

Either based on the data obtained by the sampling and risk assessment (curative) or by scheduling and planning (preventive), product applications occur. These applications can be of different nature, usually spraying applications are done.

These spraying applications can be done through a central greenhouse spraying system, using a distributed system where the applicators are then connected, or a local spraying system, with use of a mobile applicator, either with a backpack pump or a mobile spraying tank using an electrical or fuel powered pump [24].

BCA

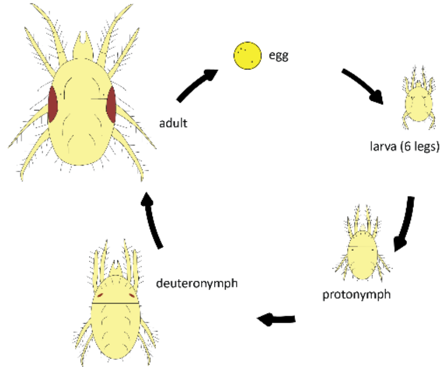
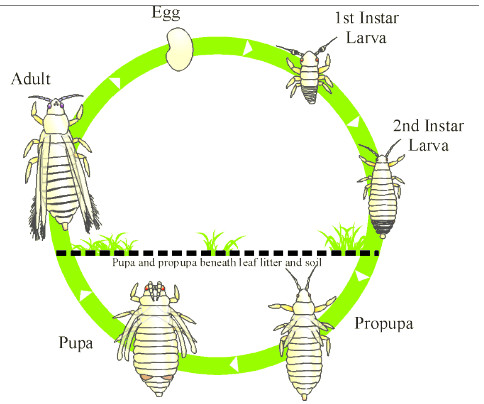
As with above, BCA releases are of the upmost importance. Managing wise BCA installation is one of the most important steps, as it normally needs a determined amount of time so they can build up a population capable of handling the crop enemies [13,14,16,24]. Application is then evaluated by sampling, either attacked leaves, of enemy counting and monitoring. Lastly it is important that chemical applications must be correct and compatible so BCAs can be maintained and avoid premature failure.

Main pests in the greenhouse roses are spider mites, which usually predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus* are used [8,15].

1.5.3. Common rose pests and diseases

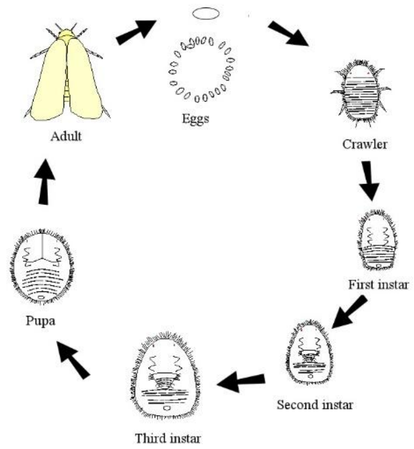
Roses have a set of typical pests and diseases. A pest is defined as a plant or animal which is detrimental to crop production, either in yield or quality, a disease is a pathogenic or environmental/physiological problem, which too can create a broad range of problems in our crop [24,40].

Rose pests[1]

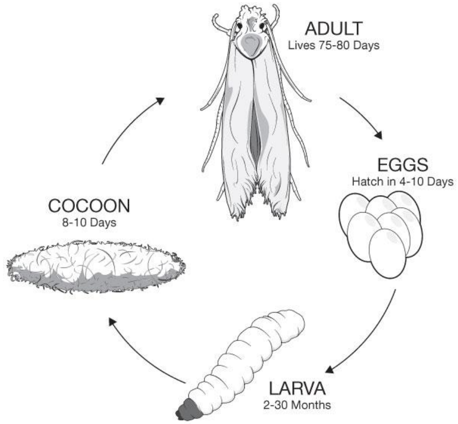
Pest Picture	Pest description	Pest Life Cycle
	**Spider-mite**: The most common is ttwo-Spotted and Red Spider Mite. They live on the underside of the leaves, puncturing them for feeding, damaging them. Difficult chemical control due to resistance build-up	
	**Thrips**: small yellow insects. Pierce plant surface causing scars, damaged leaves and defective flowers. Biological control is preferable as it quickly creates chemical resistance.	

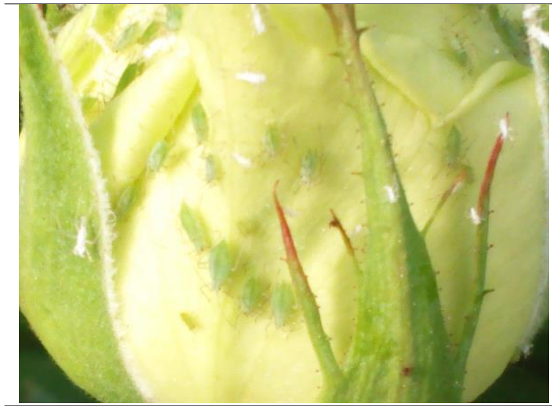
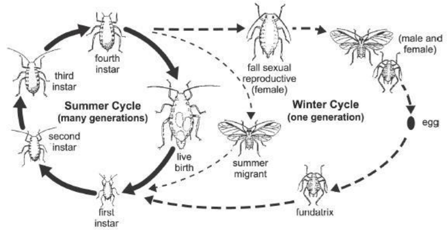
[1] Pest Pictures “Thrips”, “Catterpillar”, and all Pest life cycle adapted from [24], Pest pictures “Spider-mite”, “White-fly” and “Aphids” are original

White fly: small winged white insects that feeds by piercing the plant. Their excrement's (honeydew) attracts other pests and promotes fungus attacks. Biological control is preferable as it quickly creates chemical resistance.



Catterpillar: There are different species, but the most common include the fruit moth. It is the caterpillar stage that damage the plant by scraping the underside of the leaves. While growing they start eating the whole leaves, reducing photosynthesis and crop yield and quality. Biological or Chemical control.



	**Aphids**: small insects with different colour patterns, normally wingless – but can occur. They damage the crop by piercing the plant and produce deformed leaves or flowers. As with the whitefly their excrement's sugary substance attracts other pests and promotes fungus attacks. Chemical and Biological control is available and have good results	
Other	**Nematodes, Mealy Bug, cutworms**	

Rose Diseases[2]

Symptoms photo	Disease description and information
	**Powdery Mildew:** Fungal disease with a powdery White or grey spots. Before clear visual symptoms, leaf curling can give away its presence. Severe infection lead to chlorotic and dead leaves. Favoured by warm and dry climate, but needs high RH for spore germination. Control done by infected plant parts removal and destruction, but chemical can be highly effective, such as the use of sulphur containers. Can be prevented by pruning and favouring good air circulation and/or resistant or tolerant varieties.
	**Downey Mildew:** Fungal disease, with intercellular mycelium development. It shows as reddish, purple or brownish irregular spots on the surface. Favoured by moist and cold conditions with severe damage on the plant yield and quality. Pruning, removal and destruction of affected material is a must, with air circulation must be assured in order to reduce infection probability. Chemical control is very effective.

[2] Diseases pictures "Downey Mildew", "Botrytis" and "Agrobacterium" adapted from [24], Disease pictures "Powdery Mildew" and "Blackspot" are original

Botrytis: Fungal disease on leaves, stems and flowers appearing as brown dry material. Favoured by highly humid climate conditions. Can occur only during vase life. Pruning, low RH and air circulation can control disease, has well destroying affected material. Chemical control is very effective.

Agrobacterium: Bacterial disease, appears as tumour, mainly between roots and stem junction above soil level. Contamination normally due to wounds done by insects, nematodes or wrong crop maintenance and handling. Prevention is done by removing attacked material, use of good quality clean material and good handling practices.

Blackspot: Fungal disease shown as dark spots in the upper side of the leaves. Chlorosis occur around these spots. Pruning, density control and good air circulation avoid blackspot.

1.6. Harvest

Harvesting is done by cutting the stems at opening stage of the flower, which differs from variety to variety. Cut stage should be uniform and its judge as a quality factor in the market, this often results in several cuts along a period of time, in the same plants in a, during harvest in order to create batches of uniform cut stage [24,28].

Handling of cut flowers should be done with care as symptoms are not always visible, and often only a problem when the product reaches the "market shelves". Problems such as botrytis can occur, thorn and/or damaged petals, pressure damage, flat buds, neck damage [24]

1.6.1. In-house Transport

After harvest, flowers should be handled with care, preventing damage (poor handle, hit walls or doors) and dehydration (transport timing and direct sun light). Use of net jacks to accommodate flowers and trolley cars for transport is standard in most of greenhouses (figure 3) [24].

Figure 3. Transport material used during harvest in this study's workplace. Use of net jacks for extra protection. Original photos

1.6.2. Pack house

The place where the flowers receive a mild preparation for storage and/or transport in order to retain the optimum state of their proprieties [24].

Preparation

After harvest stem are prepared for market, as seen in fig.4 [2,24,25]:

- Defoliation of extra leaves from the lower section of the stem

- Grading the stems in order to achieve a standardization between bunches, with similar flowers in length and aspect (colour and cut stage)
- Trimming the lower section of the stem to give the bunch a homogeneous size
- Packaging, protecting the flowers. This could be in plastic wrap or in cardboard boxes. In any situation flowers should be managed with care, and, in case of plastic wrap, have space so it can be in contact with the water.

Figure 4. Stem preparation done after harvest in this study's work-place. On the left the leftovers from trimming and unseleable flower discarding can be seen. On the right stems are grouped accordingly its size. Original photos

Bucket

Prevention from dehydration is an important step in post-harvest rose maintenance in order to preserve vase life. The use of buckets in the pack house is a common practice for storage, and roses can remain a good deal of time in them, so good sanitary practices are demanded to keep the product in good conditions [24].

Good clean process before using them and a post-harvest solution (PHS) when storing the flowers are important. PHS is used to promote water uptake, while maintaining sanitary conditions, reducing bacteria growth and avoiding rising of phytosanitary problems. There is a wide variety of chemicals as stated in table 9

Table 9. PHS composition. Can have one or more of the described chemicals. Adapted from [24]

Chemical	Notes
Aluminium Sulphate	*Lowers pH* *Precipitate dirt*
Chlorine	*Bacteria management* *Solutions need refreshment as it loses effectiveness by chlorine evaporation into the air (harmless)*

Citric Acid	*Lowers pH*
STS	*Ethylene inhibitor, reducing senescence*
Wetting Agent	*Facilitates water movement trough xylem vessels*

1.6.3. Cold Storage

After preparation, roses are then stored in a cold room with required temperature of 2ºC – ensuring they will not freeze. These temperatures assure minimum respiration rate, and low heat generation. This will maintain optimum proprieties before final transport to the market supply chain [24].

1.6.4. Transport

Done in cold trucks, with temperatures between 2ºC and 5ºC, with the same purpose as the above. Assuring product quality is important, and so it is important to have quality service in this department, or all the previous work could have been in vain [24].

1.7. Market

1.7.1. Market data

By 2012 Europe produced over 21 thousand million euros in flowers, Netherlands being the top producer with 31% of those numbers and Portugal with a humble 2,3% representation with about 850 greenhouse holdings of the total 61360 holdings (outdoor and greenhouses) all-over Europe [2,3,25]

Cut flowers imports value, representing 77,1% of floriculture imports, was two times superior to exports, being the only one with a negative trade balance – near 575 million euros, although complete floricultural market show an overall positive trade balance – 313 million euros, with bulbs and corms (tulips, orchids and others) having the best contribution to this result [3,41].

Our imports come mainly from Kenya, representing 25,9% of all our floricultural market imports, with 30.7% of cut flowers alone, Ecuador falls behind in second place with only 12,6% [1.3,41].

Russia is Europe main export destination, generating 24,2% of our floriculture market exports revenue, and 42,6% in cut flower market alone – representing near 275 million euros. Netherlands is Europe main exporter being accountable of over 56% market share in all floriculture, and by far the largest exporter of cut plants with 78% market share [1.41].

Roses are among the highest selling cut flower products in the Netherlands, being “Rose Large” the largest selling flower with near 357,5 million€ revenue, with almost every type of rose having some kind of growth in sales figures in the last 5 years [42].

In terms of production, China has by far the largest are of rose production with over 10 thousand ha [2], with the second being Ecuador with a bit under 4 thousand ha. Netherlands is the European country with the largest area, being just the eighth worldwide, with only 407 ha in 2012, but keeping up in demand due to its exclusive varieties [1.3,41].

1.7.2. Import Market operation

Since import plays such an important role in the rose market it has been well organized and there are a number of ways to get their importing done [2,24,43]:

1. **Auctions**: Generally purchases done by export wholesalers, but also, in smaller scale, by florists. The main way cut flowers reach Europe wholesalers and retailers. Auctions are held by entities who manage the reception and unpacking of the imports.
2. **Agents**: Agent do the product receiving and manages selling either using the auction system or directly selling the product to Wholesalers. Their skills help growers less connected or act as a commodity service, leaving the original grower without the hassle to leave the product in the auction system.
3. **Wholesalers**: They get the roses either by the previous channels or directly from the grower. They create a network of distribution channels to the retail or florists.
4. **Flower providers**: typically they get their flowers from wholesalers, sometime having exclusivity over a wholesaler in order to get a steady and stable flow of product into their retail chains, usually the Supermarkets.
5. **Florists**: Traditional florists are usually the main hubs for retail distribution of roses in Europe. They usually offer more services than a just selling flowerers
6. **Supermarkets**: Increasing their share over the latter years, they offer convenience. Classic supermarket strategy is to offer competing prices hence getting their own share of the flower market. Other supermarkets decided to differentiate their service and changed their strategy towards quality and offering value-added products. This however demands for uniform products following a set of specifications and proprieties, not only on quality but also on vase life guarantee. Most of these supermarkets are situated either in the UK, Netherlands or Germany, as this service is not well explored by the rest of Europe.

1.7.3. Value and Quality

According to FloraHolland [43], roses are valued according to a specific requirements. A batch must be free of growth defects such as: Flat buds, Grass hearths and Crooked necks; then they are graded according to the factors in the table 10. Cut flowers have three quality groups: A1. A2 and B1. depending on the quality and grading.

In the Netherlands growers are also graded by a reliability index (BI) for a quick and good measure of their own quality; this index is built upon the information of the last 100 lots and it's given in a percentage scale. The lower the scale, the higher the need for an in depth evaluation of the product; a higher scale rewards higher sale price.

Table 10. Rose quality factors. Adapted from [24,43]

Variety	Uniformity of bud size per bunch
Size of buds	Colour and quality of leaf
Ripening stage at cutting	Free from chemical deposits and water-marking
Uniformity of bud-opening stage	Free from pests and diseases
Colour-brightness of flower	Packaging
Bud damage	Overall appearances
Uniformity of stem length per bunch	Temperature of flowers on arrival

1.7.4. Size and packaging

Normally roses are traded in cardboard boxes, and often without plastic sleeves in order to avoid humidity. After transport they are usually repacked in plastic containers (buckets) and grouped in bunches of 10 or 20 stems, with even level of flower buds or, at maximum of two layers. Stems shorter than 45 cm must be packed in smaller containers [24,42,43].

Labelling must follow a set of guidelines as well with “Supplier number and name”, “Variety name”, “Grading Marks” and a set of additional information when needed for direct trade (i.e. barcode, selling price, etc.) [24]

1.7.5. Good agricultural practices data

Social attention towards sustainable agriculture procedures have been growing among costumers, and rose market is no different. Different certification data is available such as MPS (ABC, SQ, GAP, and others) covering environmental performances [44], social issues and Good Agricultural Practices; and GlobalG.A.P. which has been a growing common standard for supermarket sales in some countries in Europe [24].

2. *Tetranychus urticae*

Two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a very small chelicerate that hosts nearly 800 plants species from vegetables and fruits to a wide variety of ornamentals and flowers. It's the most destructive phytophagous specie within the family of the Tetranychidae [45].

T. urticae is probably the main greenhouse roses (Rosa spp.) pest with estimated losses of 4500 dollars per hectare. Even in a reduced number they can do important damage; they are a year-round pest under warm greenhouse conditions [8,9,46].

2.1. Distributions

It is considered to be a temperate climate zone species also found in the subtropical regions. As such, are common in greenhouses, which offers them a habitable ambient, overcoming the different regions climates. This results in a rapid population increasing which tend to favour their high reproductive capabilities, being a common problem for protected crop growers [7].

2.2. Description

Oval, and with about half a millimetre can display different colours: brown, red-orange or, the most common, pale green, in some cases a bit translucent. This translucency often appears as the mite having two big spots – hence the common name Two Spotted Mite; this spots are just their body contents. Females are bigger, with a dozen pairs of dorsal setae. Hibernating females are orange-red. Males are smaller and display a caudal end elongated [47,48].

2.3. Life Cycle

T. urticae life cycle consists in three immature forms and a final adult. The first one, the larvae, hatches 3 days after its egg was laid, which was attached to a fine silk web. Post this stage there are two nymphal stages, protonymph and deutonymph, before the Adult.

Development of the forms depends on climate, but optimal conditions are met at near 27ºC, taking twenty days to complete their development [48].

Females often have a life span of two to four weeks and are able to lay hundreds of legs over time. Overwintering females often hibernate in ground or under the wood of older plants – explaining why, even with severe pruning in some crops the mite still remains and are able to redevelop their population [48].

2.4. Agro-economic Importance

T. urticae severely impacts the plant productivity by reducing the active photosynthetic functional area and favouring leaf abscission due to their phytophagous feeding using piercing-sucking mouthparts.

They penetrate the plant tissue, the underside of plant leaves, damaging the leaves and leave a typical yellowish scar (chlorotic spot) and necrotic spots in stages of advance leaf damage. It was estimated over 20 cells are destroyed per minute by the mite.

In time these scars covers the whole leaves, modifying physiological process such as photosynthesis, growing, flowering and fructification, causing major leave chlorosis leading to defoliation; all this results in a weaken plant with reduced life span and limited capabilities.

This is critical, especially on ornamentals where the crop value is obtained by its appearance, but the deficient development of the plant is of the upmost importance in any crop [19].

T. urticae is one of the most critical pests in a wide range of protected crops worldwide, contributing to high economical losses for the producers every year. It causes severe problems to a wide range of crops, from ornamentals vegetables, cotton, maize, flowers, legumes, vines, citrus to trees; both on outdoors fields and greenhouses [8].

2.5. Pest control on roses

Pest Control is normally based on the use of acaricides and insecticides with different active compounds, although, due to its high reproductive capability, keeping numbers under the economic injury level is difficult and a proven problem to take on consideration when doing risk assessment.

In time, use of natural enemies with biological control was proven to be the advised method to go for. Predator mites *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus californicus* (Acari: Phytoseiidae) (sin: *Amblyseius californicus*) are usually chosen for this task, have shown some of the best results in *T. urticae* control.

2.5.1. Chemical control

Acaricides plays an important role controlling *T. urticae* populations with a large number of compounds with different chemical structure and mode of action are used worldwide against *T. urticae* [13,18]

The enhanced reproductive potential of this specie, along with a very short lifecycle and an arrhenotoky capability leads *T. urticae* to rapidly creating resistances to all sorts of chemicals; this resistance has been an object of study with several publications points towards an advanced resistance mechanism [10.11]. Exposure of the *T. urticae* to diverse pesticides to maintain numbers below economic threshold have further increased its resistance to different compounds whether in greenhouse or outdoor crops [10.20.49].

This is striking to be an increasing problem, mainly in greenhouses and especially on roses, where the environment favours the resistance: the climate is normally optimum for *T. urticae* development, an increased frequency of applications, and the extended growing season typical of greenhouse production [10.11.20.45,50].

Common compounds have been reported to often fail on their tasks to control the pest, with a list of over 90 reported active ingredients by the APRD (arthropod pesticide resistance database). In the majority of cases multiple treatments were done and overdoses were common, which further increased the probability of resistance on *T. urticae*. Some studies [8] even stat that chemical control on greenhouse roses was next to impossible due to the high resistances registered and the phytotoxicity of some of the products used.

Khajehali in “*Acaricide resistance and resistance mechanisms in T. urticae populations from greenhouses in the Netherlands*" reports high percentage of resistance to older chemicals in the market. The commonly used Abamectine and Milbemectine are documented to being the ones that the pest most resisted, with its use being unadvised for its low effectiveness. Remarkably, many of the strains used, even newer, were already resistant even to new active ingredients such as cyflumetofen.

This resistances are the result of prolonged genetic selection on isolated populations over two decades of pesticide use [10].

On the other hand Spiromesifen and etoxazole show some positive results with high grades of success as there are less resistance to these. Although seeming to be a good trade-off, it is highly recommended in a chemical control program to avoid any kind of overdoses and intercalate different active compounds is advised in order to reduce resistance developing in further generations, especially in greenhouses [9,51].

2.5.2. Predatory beneficial mites, Biological Control Agent

In response to the various control problems from the chemical strategies, biological control using predatory mites has gain some popularity near the greenhouses roses producers worldwide [8].

Preliminary trials demonstrated predatory mites, *P. persimilis* releases into greenhouses would be an effective long-term control of *T. urticae* [18,19].

In time, though, effectiveness would be reduced and follow up studies concluded that predatory mites should be used with other control methods, in integrated pest management, such as use of horticultural oils and synthetic biochemical acaricides for optimum results [8,16,19].

N. californicus has been reported to exhibit higher ability to detect its prey on leaflets, and thus been shown to be a promising natural enemy. Additionally it has shown good persistence with low pray densities agroecosystems, feeding on other resources minimizing starvation. Many growers use pollen as food resources in periods of smaller pray populations [15,17].

Many assays have been done using predatory mites, and in most of these works the predatory mite adaptation process has been shown to be a fundamental step in order to achieve good results [13,14].

A good population of predatory mites should be installed in the crop before *T. urticae* populations become out of control. Then a relation between predatory mite and the pest is of the upmost importance, as the predatory mite will dynamically control its population over the numbers of *T. urticae*. Strategies can be beneficial: leaving a small number of the pest in the “maintenance lower layer” of the roses, working as a refuge, offering a feeding source for the natural enemies [14]. [15] Shown a good initial ratio to be between 5:1 to 7,5:1 *T. urticae:N. californicus* ratio.

Croft and Hoyt had already reported in the past that less than 10:1 *T. urticae:P. persimilis* was the optimum value, although Hamlen and Lindquist found ratios ranging from 4:1 to 20:1. on moderate and higher infestation levels respectively [14,46,50].

2.5.3. Control Strategies

Because economical and efficient control strategies that do not involve pesticides have not yet been developed for most of the pest of roses, biological control of *T. urticae* must be accomplished in the presence of chemical applications for control of other pests. As such in presence of chemical applications, biological control of *T. urticae* has to be achieved with a selective use of pesticides taking into account their toxicity to the predatory mites [46,51–53].

2.6. Risk Assessment

Roses, as with almost every crop host to *T. urticae*, should be regularly monitored for pest presence during the productive period – due to cut flowers nature normally this means throughout its entire life. No formal action threshold/ET exists but there are some guidelines from previous studies [13].

There has been found that densities between 10 and 50 mites per leave leads to a reduction in 17% to 26% of the plant stem, and although we can find a suitable number to take measures, pest control is normally as successful as soon we can control its population development [14,46].

Although some methods can give us an accurate data, and be even helpful for risk assessment, they are methods too complex for daily usage; and are rather time consuming for the farmer to use and maintain an up-to-date data for risk assessment and decision making.

II. Methodology

1. Essay Workplace Set-up

The present study was done in association with FLORALVES, a dedicated flower producer business situ in Fajozes, North of Portugal with over two decades of experience. Along with Roses, they produce Gerbera, Alstroemeria, Lisianthus, Sunflowers, Lilium, Carnation, Sea-lavander and Proteas.

A dedicated greenhouse is assign to rose production, partially built with comunitary funds from a PRODER program dated back in February, 2011. This was the work place of our essay.

1.1. Greenhouse specifications

A typical Dutch Venlo glass-type greenhouse (KUBO, NL), with near 1 ha of productive area.

The glass is wind resistant protecting the greenhouse interior and avoids water condensation, and water dripping into the flowers; a thermic screen layer is used to maintain the lower temperatures above negative, but needs maintenance as it is washed away with rain, common in this region. The use of plastic material inside the greenhouse works diffusing the light rays.

The greenhouse is capable of heating control using a boiler system, maintaining an ambient temperature of 20ºC, produced gas from the combustion process to heat the boiler can be used to inject CO_2 into the greenhouse. The heating system was not used during this year offering a gradual harvesting for market demands and reducing Botrytis infection.

Climate control, with control over vents and fan circulation along with temperature (min, max and average), RH, CO_2 and wind speed is done using OPTICLIMA CL600 greenhouse climate management system.

2. User friendly non-invasive *T. urticae* Risk Assessment methodology on Greenhouse Roses

2.1. Methodology Proposal Background

Monitoring and forecasting crop condition is a prime necessity for risk acessement and early diagnosis. This ensures the best control over the production needs and present and future situation, whether on pest and decease control or decision making [36]. The easier this is achieved the better, simplyfing interpretation on the easy to use information gathered.

During review and preparation phase for an on going MSc thesis on "Comparation between diferent *Tetranychus urticae* control methods on Greenhouse *roses: Solving Floralves case-study*" it was noted, while lab or destructive methods were the common methods for evaluation, the lack of a simple, user friendly, non destructive and in locus method for risk assessement and easy to use pest monitoring [8,20].

The two-spotted spider mite, *T. urticae Koch* (Acari: Tetranychidae), is one of the most important pest species responsible for significant yield losses in many horticultural, ornamental, and agricultural crops world wide, being a major pest of greenhouse roses. Even a small number of individuals can severely injure the host plants as they prosper under warm greenhouse conditions [8]. Their fast chemical resistence mechanism helps achieve this unwanted status [10.54].

Monitoring and forecasting crop condition is a prime necessity for risk assessement and early diagnosis. This ensures the best control over the production needs on decision making (Boller et al. 2004). The easier this is achieved the better, simplyfing interpretation on the information gathered.

Cut roses farmers doesn't have a T. urticae risk assessment methodology to help them in decision making for a better pest control.

It is needed to evaluate T. urticae damages and find quantification techniques for risk assessment in rose production, to correlate the level of the pest with losses and, finaly, to propose an easy and reliable method for estimating the risk for T. urticae in grenhouse roses

2.2. Set-up

2.2.1. Biological material

Roses were produced in hydroponic system with coconut fiber substract in a greenhouse located in the Northwest region of Portugal (Fajozes, Portugal) known to have a severe presence of *T. urticae*.

Leaflets from Rosa hybrida cv. “White Naomi” and cv. “Red Naomi” were used. A total of 138 terminal leaflets were random hand-picked 82 from Red Naomi and 56 from White Naomi

2.2.2. Observation material

All the biological material were observed, in lab, with the resource of a zoom stereomicroscope Nikon SMZ1000 using a standard set of 10x eyepiece and an 1x objective, totaling an magnification of 80X. This was coupled with an HD Color Camera Head Nikon DS-Fi1. enabling digital photo acquisition right from the stereomicroscope; this was done using the incluided NiS elements BR 3.2

2.2.3. Observation analysis

Each individual leaflet was observed, the number of mobile forms (adults and juvenile stages) and eggs were registered and a level of intensity was proposed. Moreover the presence of exuviae was registered.

To validate proposed scale, terminal leaflets were randomly chosen, 20 in each sample, 10 from maintenance layer and 10 from production layer, over a course of 15 weeks, twice a week, in a total of 8040 leaflets observed.

Risk assessment was then obtained by the average of the scores, obtaining an overview of the current intensity of the pest in area sampled in the greenhouse.

1. Comparison between three different control methods for *T. urticae* on Greenhouse Roses

1.1.1. Time span

Field work began at 16 March 2015, 3 weeks before the sampling began for preliminary essays, methodology set-up and testing.

Monitoring and Sampling last for 17 weeks, starting at 7 April 2015 and ending at 31 July 2015, with 2 periods separated by a “first harvest”, which was in fact a maintenance procedure with crop restructuration practices. The sampling ended with a second harvest and a control method change to better suit sampling results.

1.2. Plant material and growth conditions

One rose cultivar (*Rosa Hybrida* L. cvs.”White Naomi”) obtained from a commercial propagator (Schreurs, NL)[3] planted in a soilless, hydroponic system using an in-bag substrate from a commercial formulation (Horti Pro, NL).

“White Naomi” was chosen for its higher infection ratio reported by the farmer.

The study focused in four different sections for sampling and comparison essay.

1.2.1. Plant Density

Plants were grown as part of the commercial decisions from FLORIALVES, initially in a 16 rows per section with 4 plant per substrate bag, totalling a density of 8 plants per square meter.

Part of a greenhouse restructuration in order to try to help in the pest controlling as a cultural control, density was reduced to about 6 plants per square meter with 8 rows per tunnel, but 6 plants per substrate bag.

1.2.2. Essay preparation

Along with density reduction a severe pruning was done, removing all the canopy area and plants were treated with an oil (**Cultaza** OLEX) in order to eliminate to the possible extent *T. urticae* individuals that can remain in the woody part of the plant.

2.3. Pest Control methods

Initial pest control methods were chosen with three different treatments as seen in the table 11. all based on commercial formulations.

[3] Online cathalog at: http://www.schreurs.nl/

Table 11. Initial Treatment used Overview

Treatments	Commercial Name
Standard Biopesticide (SBP)	SAPEC Boreal (*Abamectine*)
New Biopesticide (NBP)	Cultaza Serv-Mite (*biopolymers*)
BCA (Predatory mites)	Koppert SPIDEX (*P. persimilis*); Koppert SPICAL (*N. Californicus*)

2.3.1. Control methods initial set-up

Four modules in the most north-western section of the greenhouse were available for the essay. Treatments were divided as illustrator in the figure 6.

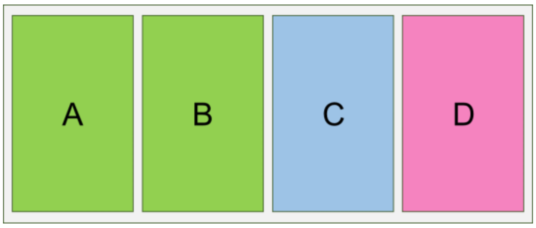


Figure 5. Greenhouse essay section division. Represented by each rectangle is the modules at disposal during the essay (A, B, C and D). A and B, received BCA treatment; C, received NBP treatment; D, received SBP treatment

The two western modules were used for the BCA releases, the third module used the new Bio pesticide formulation and the last tunnel used the standard biopesticide.

The reasons for this choosing follows:

- BCA are more susceptible to chemicals and temperature differences, as such, being that for logistic reasons the area we had were more influenced by this parameters, in order to establish good readings this method was offered two modules. This ensured a larger area to diminish possible chemical interactions, and to get a sampling area further from the greenhouse western wall.
- The other treatments followed a similar logistic pattern, as the new chemical shows no impact over auxiliary BCA, placing it next to it assured even more chemical safety
- Standard biopesticide would have advantage to be in the final module at our disposal, so it would be easier to apply by the greenhouse staff, as no interval was needed to use it because was followed by other plants that shared the same treatment.

Additional applications

All sections received after pruning an application of horticultural oil (**Cultaza** OLEX®, WP) and an ovicide (**SAPEC** AGRO Tenor®, WP); during the essay all modalities also received of “Floramite, SC” and “Sipcam Nissorum, WP” applications. The first targets eggs and the latter is specific for larval states.

All additional treatments were compatible to the main treatment selection. Compatible treatments with predatory mite were confirmed by means of Koppert own web-cloud tool offered to farmers using their products.

Predatory mites release info

Following previous Koppert protocols in greenhouses following similar variety/climate patterns, the predatory mites release ratio was defined to be 10 individuals per square meter and latter uprated to 16 individuals per square meter.

2.3.2. Control methods follow up

Control methods were adjusted over time due to different reasons:

- Standard biopesticide remained the same until the end of our study;
- New Biopesticide was discarded after week 10 in favour of the standard treatment (Figure 6). This was due to the extreme low efficacy, and a way of the producer try to recover production at that section.
- Predatory mite releases were increased to 16 individuals in response to the results, 7 weeks after the beginning of the field work.

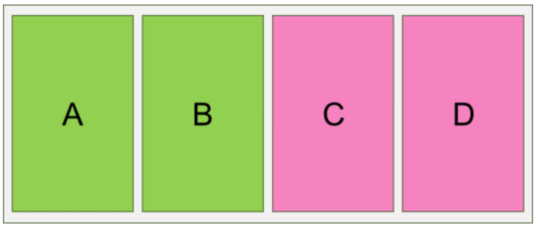


Figure 6. Greenhouse essay section division after the 10th week. Represented by each rectangle is the modules at disposal during the essay (A, B, C and D). A and B, maintained BCA treatment; C, previously with NBP (discarded), and D, were both treated with SBP

2.4. Sampling and monitoring

2.4.1. Experimental Design

Sampling was done with method described in the chapter III (1.1.) of “*User friendly non-invasive T. urticae Risk Assessment methodology on Greenhouse Roses*” (Table 13).

The center portion of each section were chosen for sampling; Each section had 8 rows: one in each section border and 6 in the centre section (a pair on the left, a pair on the middle and a pair on the right). In each pair, one row was chosen, totalling 3 rows as pictured in figure 7.

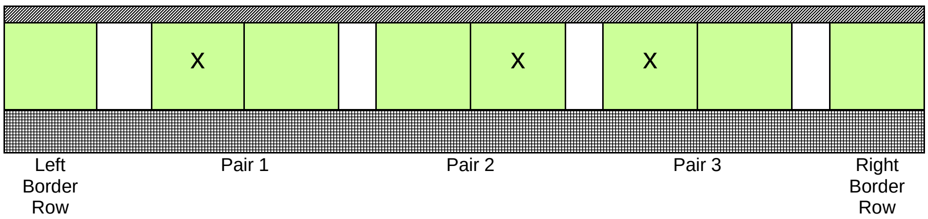


Figure 7. Greenhouse Section Overlay. The top slashed pattern figures the Greenhouse wall, and the bottom crossed pattern figures the Greenhouse pathway. Each middle coloured block figures the crop rows present in the section. For the study only one row in each of the centre Pairs (1-3) were used: the chosen ones are marked with an 'x' as an example.

Next step was dividing this in regions, an initial, near the greenhouse pathway, other in the middle of the section, and a final in the furthest point from the pathway, near the greenhouse wall; in each zone a set of flowers was chosen – for easy marking this was done by choosing a substrate bag.

Sampling was then done twice a week in each row, rotating between region (near, middle and farther). In each zone n=10 leaflets from the maintenance layer and another n=10 leaflets from the production layer were sampled, scoring them accordingly with the levels in table 13.

2.4.2. Fast monitoring for fast decision

All the values registered were inserted into a spreadsheet, and a simple mean test was done offering a quick and simple score helping decision making.

Intensity and Severity of the attack were also calculated as indicators using the following equations:

$$Attack\ intensity\ (\%) = \frac{nAttacked\ Leves}{sampled\ leaves}\ x\ 100$$

$$Attack\ severity\ (\tilde{n}\ mites\ per\ leave) = \frac{\sum leaves\ per\ class\ *\ class\ boundary}{sampled\ leaves}$$

These values were calculated twice a week and presented to the Koppert team. This was done in order to optimize the BCA control method: for releases and density numbers decision.

2.5. Quality and Productivity

Evaluated at harvest; quality was measured using both stem height and flower bud width, productivity was measure by the total count of the flowers harvested and displaying quality needed to be saleable. All the values, both from measures and counting, were separated by size categories.

All measurements were done manually with the aid of a Vernier Calliper for flower bud measurement and a measuring tape. Flower counting was done manually by the greenhouse workers while bunching the flowers. A general sampling of 30 random stems were done for both SH and FBW, and an additional 15 stem per bunch category were sampled.

Flowers that didn't show minimal quality parameters (torn or chewed petals, chlorotic leaves, too small, etc.) were discarded.

2.6. Statistical and Data Analysis

Sampling raw data, intensity, severity, pest evolution graphs and productivity were calculated and done using Microsoft Excel 2013 from Microsoft Office 2013 Suite. Treatment data, quality and productivity data were then treated using GraphPad PRISM 6 for Windows.

A "Spearmen ρ" Correlation test was used to correlate pest presence in both layers; stem layer (production layer) and maintenance layer [55].

Layer data were further compared using the Mann-Whitney test, due to the ordinal nature of our data [55], and T-Test in order to find if there was a difference during our trial.

The same test was done to compare between treatments (Standard Chemical vs. New Chemical; Standard Chemical vs. BCA; BCA vs. New chemical), and repeated for the data after the first harvest (Standard Chemical vs. BCA). The same test was applied to test differences between the production layer and the maintenance layer and to further investigate relation between them.

Harvest data, as stem weight and flower bud size data, was treated using ANOVA

Treatment effects were tested at 5% probability level ($p>0.05$) and multiple comparisons were all done using 'pairwise' configuration.

2.6.1. Presence and Intensity/Occupation data

Data gathered was compared both in intensity and occupation numbers.

Presence score was obtained by calculating the mean score in all rows for each day in each treatment. This score is in accordance with the Level Score data referred in *chapter III (1.1.) (Table 13)*. This data is given in an ordinal scale from “0” trough “3”.

Intensity Rate is obtained using the formula in chapter 2.4.2, and draws leaflet occupation numbers, obtained by scoring only pest occupied leaflets, referring to the mean number of occupied leaflets in a plant. This data is given in percentage.

III. Results

1. User friendly non-invasive T. urticae Risk Assessment methodology on Greenhouse Roses

1.1. Results

Observation of mobile forms and eggs presence allowed the division of the 138 leaflets in four groups according to the attack level (Table 12).

Table 12. Proposed level of intensity and their occurrence in the leaflets as mobile forms and eggs.

Proposed level of intensity	Nº specimens in each leaflet	Nº leaflets (mobile forms)	Nº leaflets (eggs)
0	0	57	58
1	1 – 2	10	9
2	3 – 5	13	15
3	≥6	58	66
	Total	138	138

The presence of *exuviae* was observed in 48% of the leaflets and among these, 73% were classified in level 3 in relation to mobile forms and 70% in level 3 in relation to eggs. The positive correlation between the presence of *exuviae* and the level of attack intensity is due to the fact that during previous weeks no pesticides application have been done and *ecdysis* has occurred for many individuals living on the leaflets. Although this positive relation exists, the presence of *exuviae* only indicates the previous occupation by the pest and therefore is not an indicator for risk assessment.

From the data gathered a scale was proposed (Table 13). Observations lead to conclude that one to two individuals per leaflet would be the defining point for a low level attack (1), as this range of leaflet occupation no weakening signal is shown.

In leaflets with more than two individuals effects of pest presence are perceptible, such as lesions from the piercing and sucking attacks on the plant, thus defining a medium level attack (2). Higher number of individuals per leaflet increased damage on leaflets, and generally on the host plant, to near or complete irreversible.

The feeding style of the pest would be so hard on the organism, photosynthesis capability would be compromised. It was estimated that above six individuals it would be considered a high level attack (3). Methodology was similarly done to the eggs reading.

Table 13. Proposed scale for T. urticae risk assessment in greenhouse roses

Level assigned	N. Mobiles	N. Eggs	Name	Description
0	0	0	Null	Pest absent
1	1 – 2	1 - 2	Incipient	Pest present in economic acceptable levels
2	3 – 5	3 - 5	Medium	Pest present in non-acceptable levels
3	≥6	≥6	Intense	Pest present in high levels with irreversible damages

1.2. Purposed Protocol

Evaluation of the monitoring area should be made in order to find a suitable and representative sampling. Taking an example of the scheme in the figure 8, a greenhouse with 5 rows, a suitable division with balanced sampling areas is needed. We chosen the odd rows (1. 3 and 5). We could simplify the reading and take another step: subdividing this regions, achieving 3 areas in each rows. This could be important if you know before hand the area in monitoring have potentially different rates of evolution of the pest for various reasons and want to assess it.

Sampling was done rotating between the areas in each sampling day, sugested in the figure 5d, obtaining a overall reading of the greenhouse.

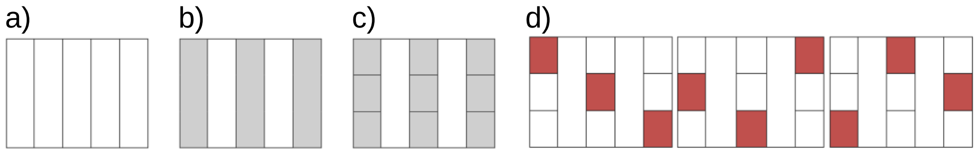


Figure 8. Sampling area division in a five row greenhouse example. Panel a: greenhouse space overall space representation; Panel b: crop lines representation; Panel c: teorical line division for sampling; Panel d: example of sampling rotation.

Sampling consistis on a regular scoring of a leaflet sample acording to the levels suggested in the *chapter III (1.1.)*. During the current essay, the levels purposed on the Table 13 (0-trought-3) were used.

Scoring is registered on-site, while sampling leaflets; in this essay 10 leaflets both from the Production layer and Maintenance Layer (n=20) were used.

1.3. Final Results

During this essay For 15 weeks, twice a week, observations were done using the proposed scale. 8040 leaflets were observed and classified on the assigned level for mobile forms and eggs, 50% for maintenance layer and 50% for producer layer. This leads to a new scale revised and a proposal methodology.

The knowledge of a strong positive correlation outcome between maintenance layer and production layer assigned level (R^2=0.86), and the fact of maintenance layer attack is always slightly higher than the production layer, allow us to restrict observations solely to maintenance layer without any increased risk.

Moreover it was observed that plants at level 2 were too heavily attacked. As such the economic threshold suggested is 1 and the level 3 is discarded.

A revised scale is then proposed (Table 14) associated with a methodology.

Table 14. Revised scale for user friendly T. urticae risk assessment in greenhouse roses

Level assigned	Nº T. urticae (mobiles or eggs)	Name	Description
0	0	Null	Pest absent
1	1 – 2	Incipient	Pest reached Economic Threshold;Treatment is needed
2	≥3	Medium	Pest above ET; May need drastic corrective treatment

This data were only available after the complete essay was done, and thus, the current study was done using the previous purposed scoring scale (Table 12).

2. Comparison between different control methods

2.1. Attack presence score and intensity rate evolution

2.1.1. Maintenance Layer (ML) sampling

Mobile Forms

In all three treatments, attack presence score increased over time [figure 9.], all reaching a high attack level. No significant differences were found between the different treatments (Average Class values: BCA = 1.6±1.0; NBP = 1.4±0.9; SBP = 1.4±1.0) , and all the three treatments reached level 3, although the NBP fell to level 2 before the harvest. Peaks seen in figure 9 graphs are mainly response to the applied treatments.

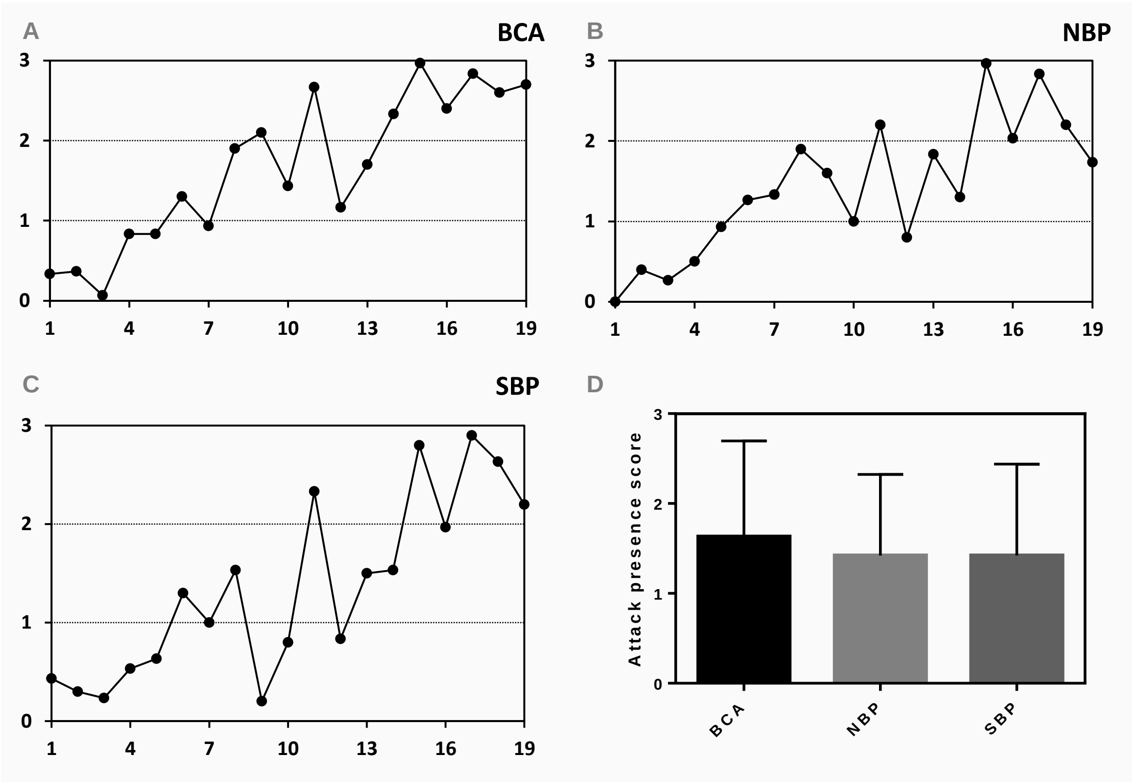


Figure 9. Mobile *Tetranychus urticae* individuals Presence Score evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effects on *T. urticae* Presence was scored during 10 weeks. Results shown in these panels were obtained by the mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values during the 10 week time period. Statisticaly differences ($p<0.05$) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

Attack intensity was also evaluated [figure 10.] and compared for differences, as further data could be gathered, but as with the presence score, no statistical differences were found.

Even though NBP drop below the level 2 score mark [figure 9.], occupation was still high, producing a negative impact for the crop and a worrying factor for the farmer (Average Occupation values: BCA = 69.1±29.5 %; NBP = 58.2±28.5 %; SBP = 57.5±31.1 %).

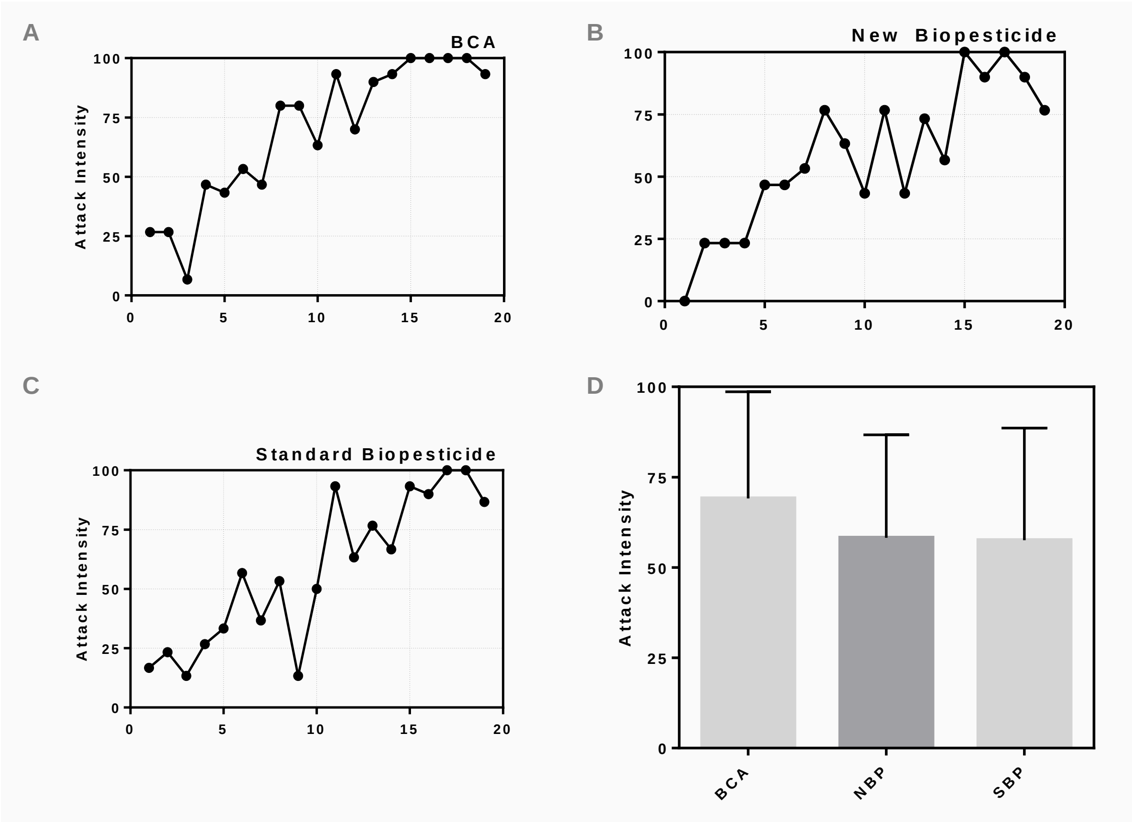


Figure 10. *Tetranychus urticae* mobile forms Intensity rate evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effects on *T. urticae* intensity rate were obtained using the formula in chapter *II.2.4.* during a time period of 10 weeks. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

Eggs

As with the mobile forms, eggs also increased over-time [figure 11], drawing brief class peaks, mainly due to brief-response to the treatments. All treatments have reached the maximum Presence Score of 3 (Average Class values: BCA = 1.6±1.0; NBP = 1.2±0.8; SBP = 0.9±0.9). Analysis found statistical difference between the values achieved in the BCA treatment and SBP (P=0.033). The latter treatment was able to contain the egg intensity at lower numbers that the BCA, this and the high peaks could be held accountable for this difference, but unfortunately both treatments ended up with high pest occupation and attack intensity.

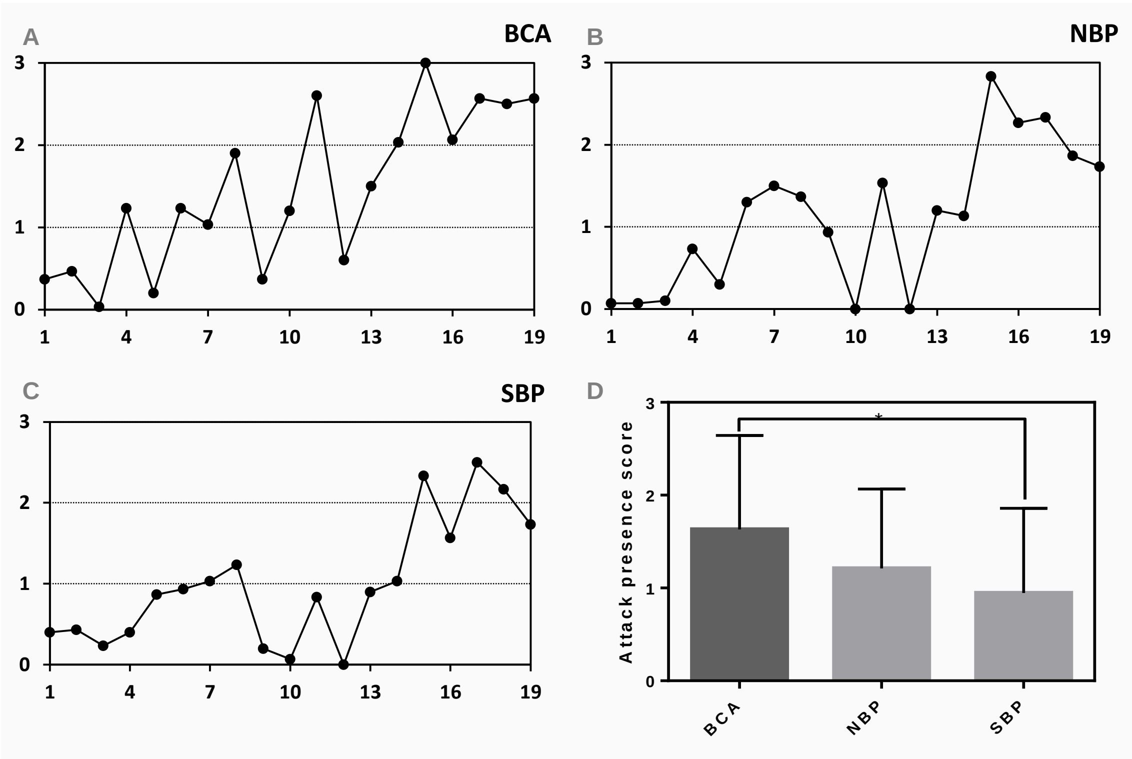


Figure 11. Pest Eggs Presence Score evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effects on *T. urticae* Presence was scored during 10 weeks. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

Shown in figure 12, occupation shown no statistical difference. The peaks in BCA are not as pronounced as in presence classification. However, even with slightly lower intensity in NBP and SBP, occupation in these were pretty high (over 70%, with BCA being the worst with 90% end intensity) (Average Occupation values: BCA = 37.7±33.0 %; NBP = 37.2±30.1 %; SBP = 34.4±36.0 %).

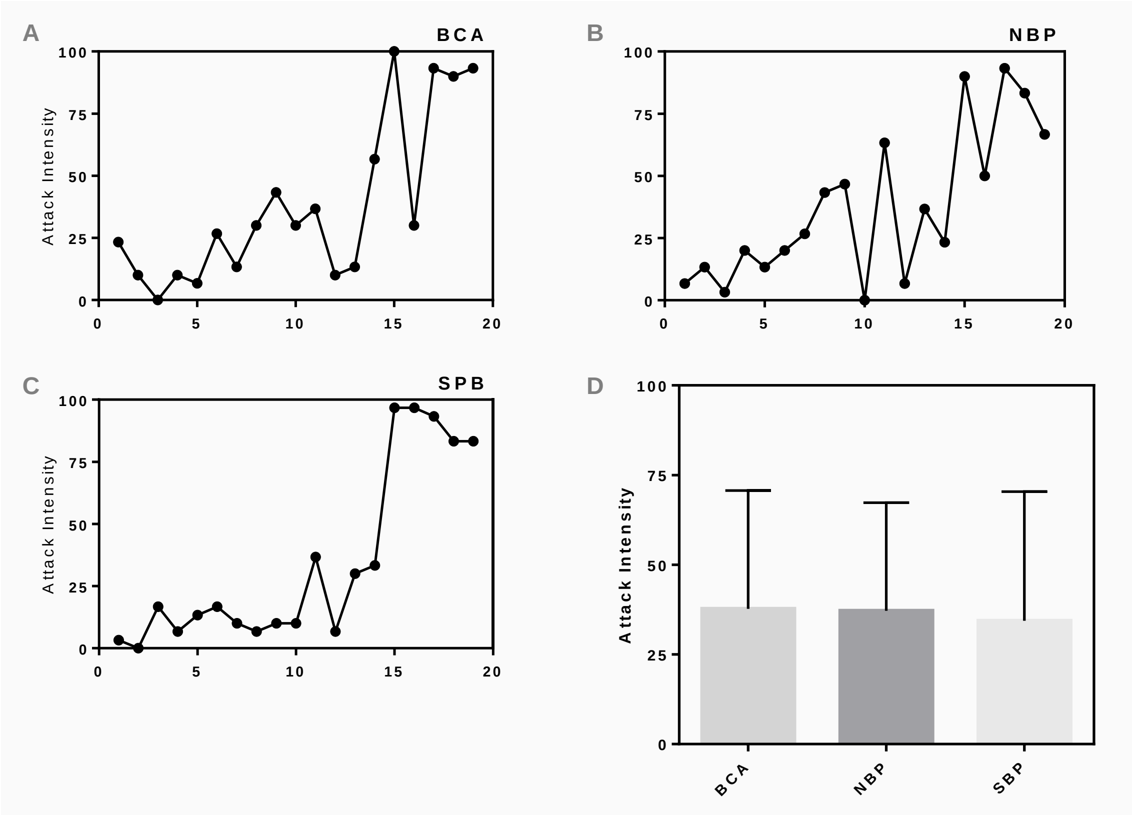


Figure 12. *Tetranychus urticae* egg Instensity rate evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effects on *T.urticae* ocupation rate were calculated from Presence data scored during 10 weeks. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values. Statisticaly differences ($p<0.05$) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

2.1.2. Production Layer

Mobile Forms

As with the maintenance layer, the production layer also follow the evolution pattern as expected [figure 13.]. The pest intensity in stems was lower than in the maintenance layer, with acceptable numbers until a peak was reached after the 7th week, presenting a lower mean throughout the weeks; this was due to the later grow of this layer in comparison with the ML. Each treatment had reached the Presence Score 3 (Average Class values: BCA = 0.9±1.1; NBP = 0.8±1.0; SBP = 0.8±1.0) No statistical significance was found in pest intensity.

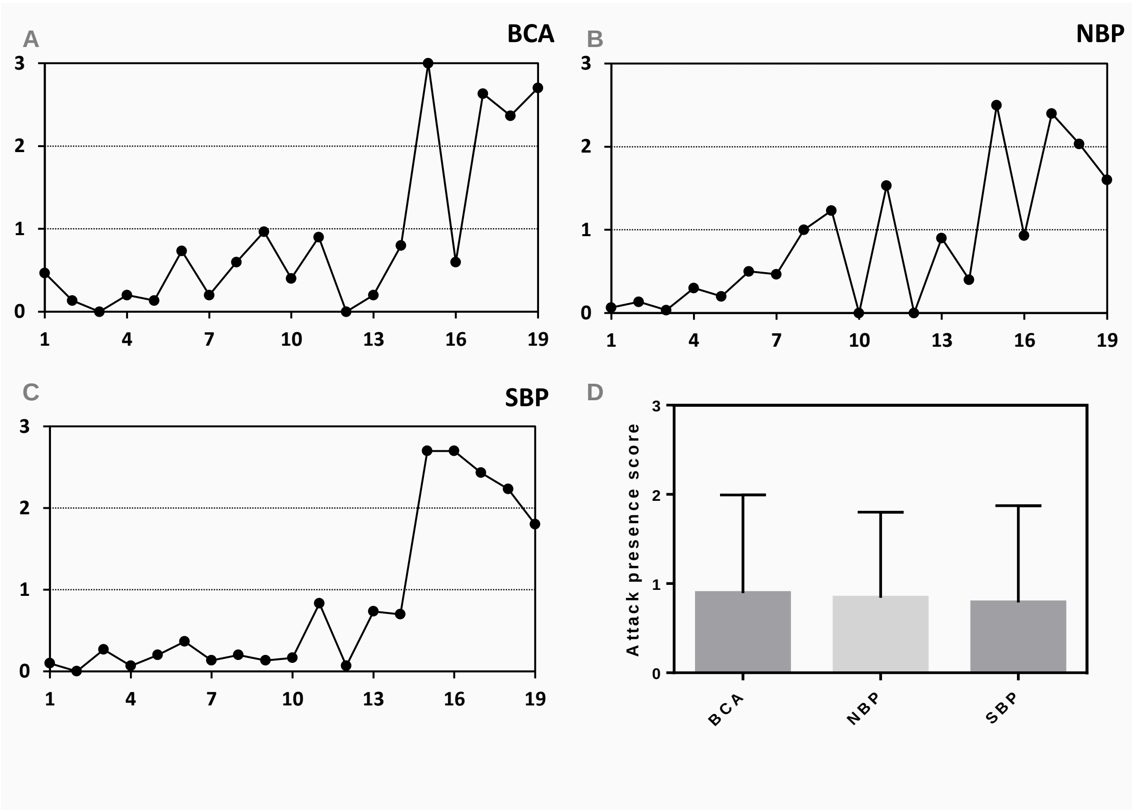


Figure 13. Mobile *Tetranychus urticae* individuals Presence Score evolution by treatment in production layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effects on *T. urticae* Presence was scored during 10 weeks. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values. Statisticaly differences ($p<0.05$) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

As before, intensity increased over time [figure 14.], but more pronounced results were found, especially between the BCA and SBP treatment (P=0.0301) (Average Occupation values: BCA = 61.1±29.9 %; NBP = 46.3±29.1 %; SBP = 40.0±27.6 %).

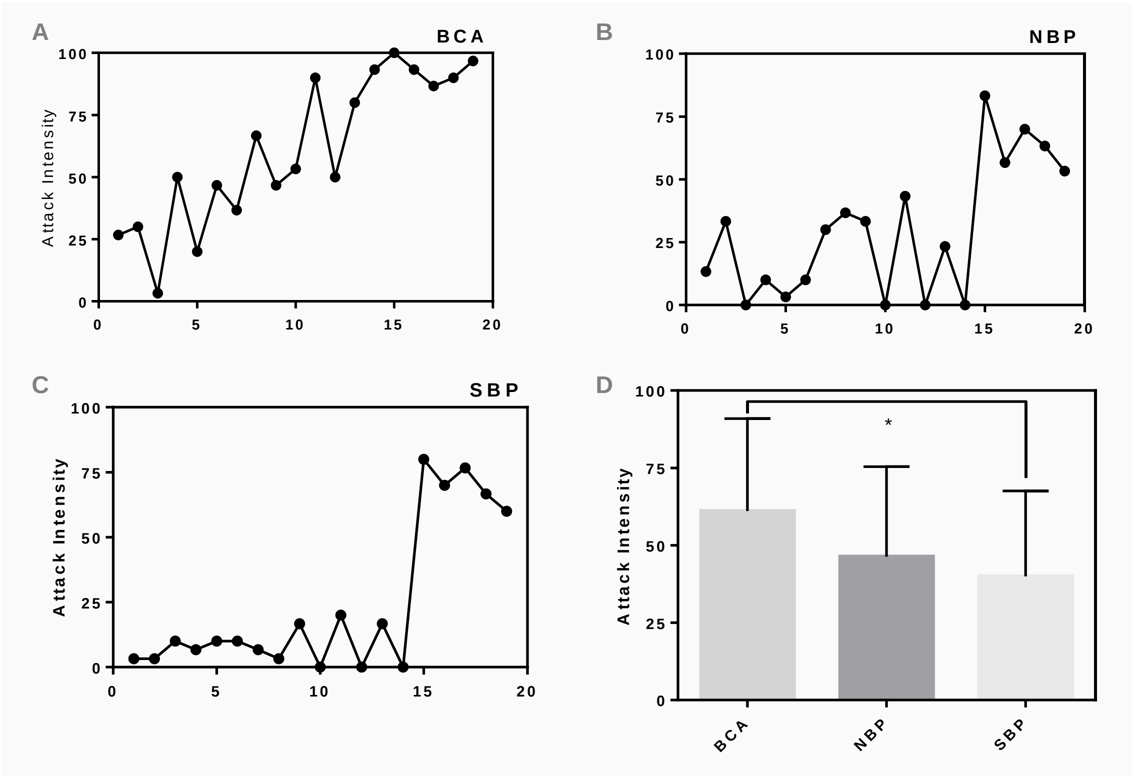


Figure 14. *Tetranychus urticae* mobile forms Instensity rate evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effect on *T. urticae* ocupation rate were calculated from Presence data scored during 10 weeks. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

Eggs

Eggs scoring evolution followed a similar pattern as with the mobile forms, with acceptable numbers until the final 4 weeks, with both NBP and SBP not reaching a Class 3. Mean-wise the values stay under the first class (Average Class values: BCA = 0.8±1.1; NBP = 0.7±0.7; SBP = 0.6±0.8). No statistic meaning was found.

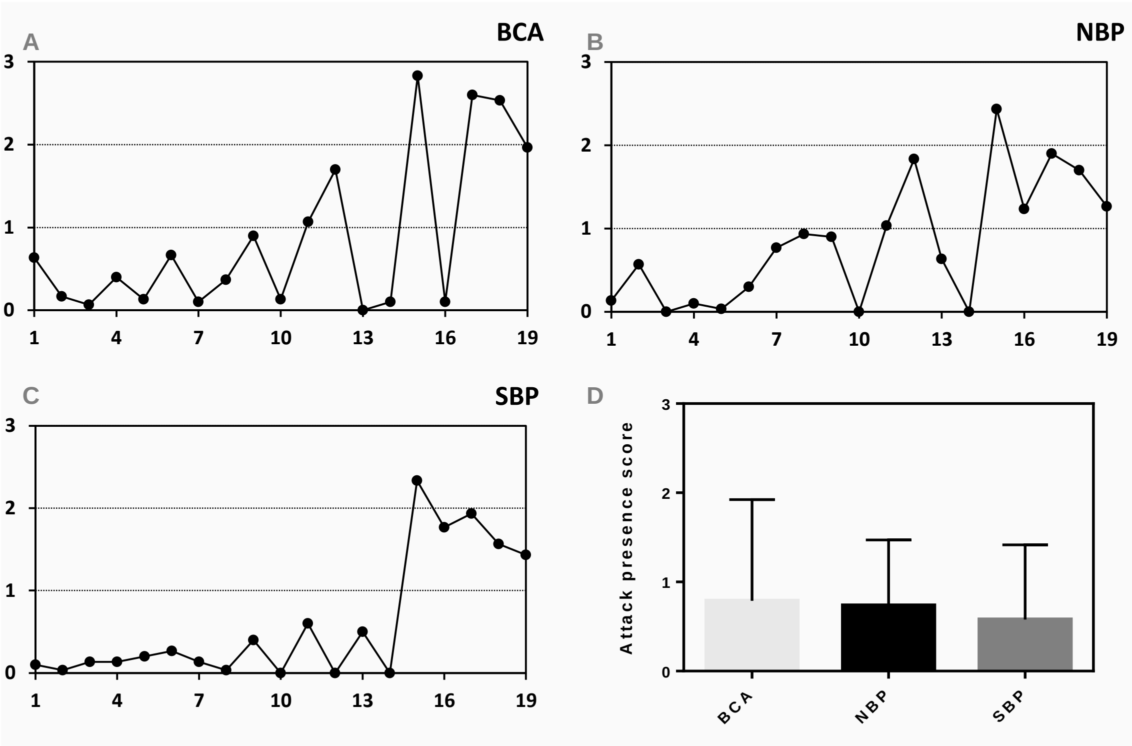


Figure 15. *Tetranychus urticae* egg Presence Score evolution by treatment in production layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effects on *T. urticae* Presence was scored during 10 weeks. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

Attack Intensity trailed the same pattern, with clear growth in attacked leaves [figure 16.] (Average Occupation values: BCA = 28.8±34.3 %; NBP = 29.6±26.3 %; SBP = 24.2±29.3 %).

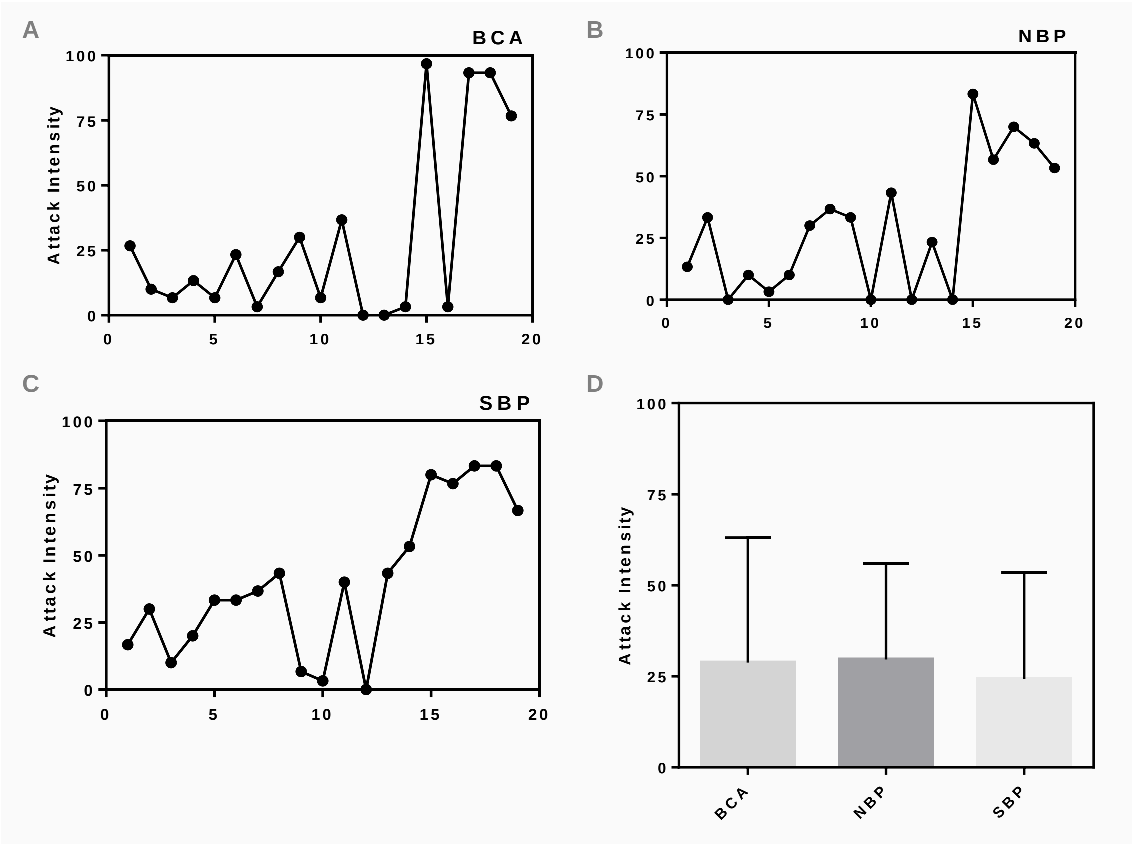


Figure 16. *Tetranychus urticae* egg Instensity rate evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; New Biopesticide (Cultaza SERV-MITE) [panel B]; Standard Biopesticide (Sapec BOREAL) [panel C] effect on *T. urticae* ocupation rate were calculated from Presence data scored during 10 weeks. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel D shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A,B and C, represents Sampling Day.

2.2. Production Layer and Maintenance Layer relation

It was interesting to understand if there was a relation between both layer of the plants in the same treatment, in other words if a certain pest score found in the bottom layer would relate to the same score as in the stems.

Correlation between layers was found in all control methods both in:

- presence score (**Mobile forms**: BCA, r_s=0.8552, *P<0.0001*; NBP, r_s=0.8859 *P<0.0001*; SBP, r_s=0.8406, *P<0.0001*; **Eggs**: BCA, r_s=0.5360. *P<0.0180*; NBP, r_s=0.8046, *P<0.0001*; SBP, r_s=0.8159, *P<0.0001*) [figure 17];
- and attack intensity rate (**Mobile forms**: BCA, r=0.9461. *P<0.0001*; NBP, r=8255, *P<0.0001*; SBP, r=0.7938, *P<0.0001*; **Eggs**: BCA, r=0.9099, *P<0.0001*; NBP, r=0.9291. *P<0.0001*; SBP, r=0.9684, *P<0.0001*) [figure 18].

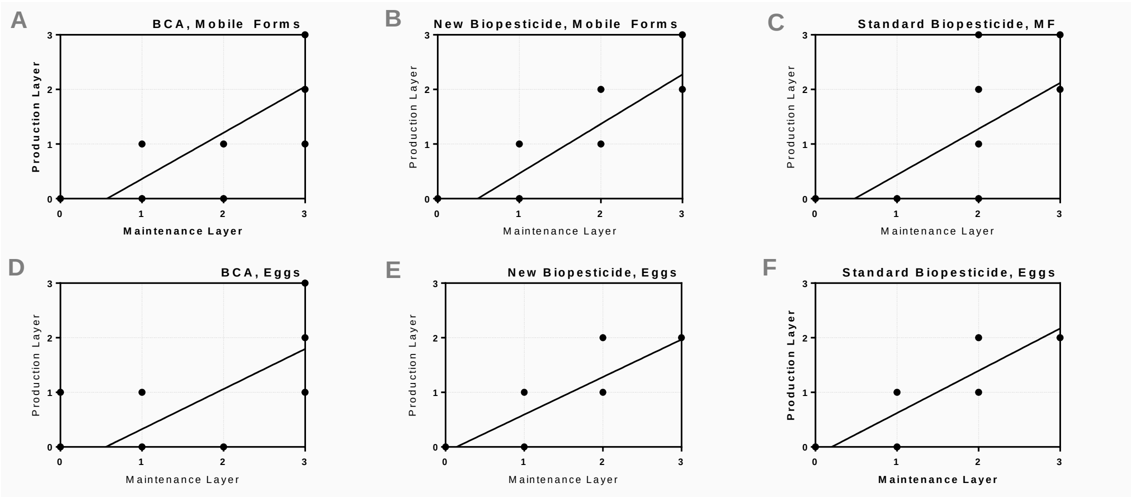


Figure 17. *Tetranychus urticae* Production Layer x Maintenance Layer Presence Score correlation by treatment. Mobile forms treated with Biological Control Agent (predatory mites) releases, New Biopesticide (Cultaza SERV-MITE) and Standard Biopesticide (Sapec Boreal) [respectively Panel A, B and C]; Egg treated with Biological Control Agent (predatory mites) releases, New Biopesticide (Cultaza SERV-MITE) and Standard Biopesticide (Sapec Boreal) [respectively Panel D, E and D] were correlated using Spearmen correlation from *T. urticae* effects on presence score data during the 10 weeks before the technical harvest. Linear representation of correlation is shown.

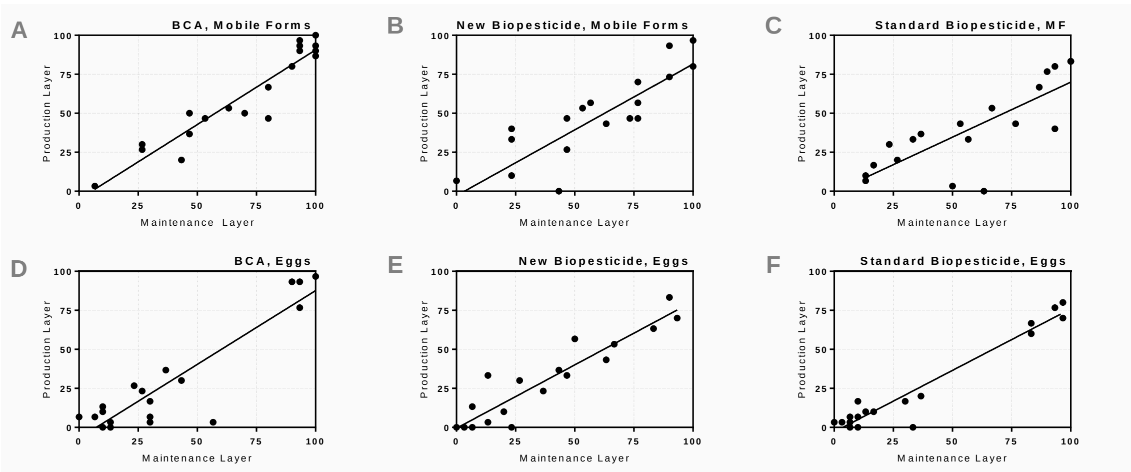


Figure 18. *Tetranychus urticae* Production Layer x Maintenance Layer Intensity rate correlation by treatment. Mobile forms treated with Biological Control Agent (predatory mites) releases, New Biopesticide (Cultaza SERV-MITE) and Standard Biopesticide (Sapec Boreal) [respectively Panel A, B and C]; Egg treated with Biological Control Agent (predatory mites) releases, New Biopesticide (Cultaza SERV-MITE) and Standard Biopesticide (Sapec Boreal) [respectively Panel D, E and D] were correlated using Pearson correlation coefficients from *T. urticae* effects on Intensity rate data during the 10 weeks before the technical harvest. Linear representation of correlation is shown.

2.2.1. Analysis

Mobile Forms

Comparison analysis between the maintenance layer and production layer showed higher values to the Maintenance layer. A statistic difference was found between the attack presence score in BCA treatment (P=0.0342) [figure 19.].

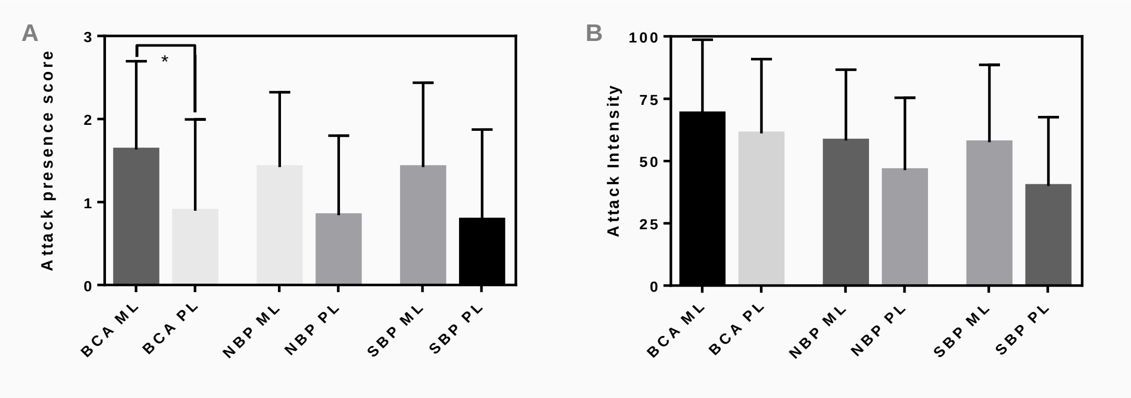


Figure 19. *Tetranychus urticae* Production Layer x Maintenance Presence Score and Intensity rate comparison by treatment. Mobile forms treated with Biological Control Agent (predatory mites) releases, New Biopesticide (Cultaza SERV-MITE) and Standard Biopesticide (Sapec Boreal) were compared by layer both in Presence score [Panel A], using Mann-Whitney test, and Intensity rate [Panel B] using unpaired t test correlated using Pearson correlation coefficients from *T. urticae* effects on Presence score data during the first 10 weeks. Statistically differences ($p<0.05$) are noted with an '*'.

Eggs

As with the mobile forms, the analysis brought the same results regarding the egg presence score with the same statistical difference (P=0.0103). Remaining values, while showing no statistical differences, in average PL values were inferior to ML.

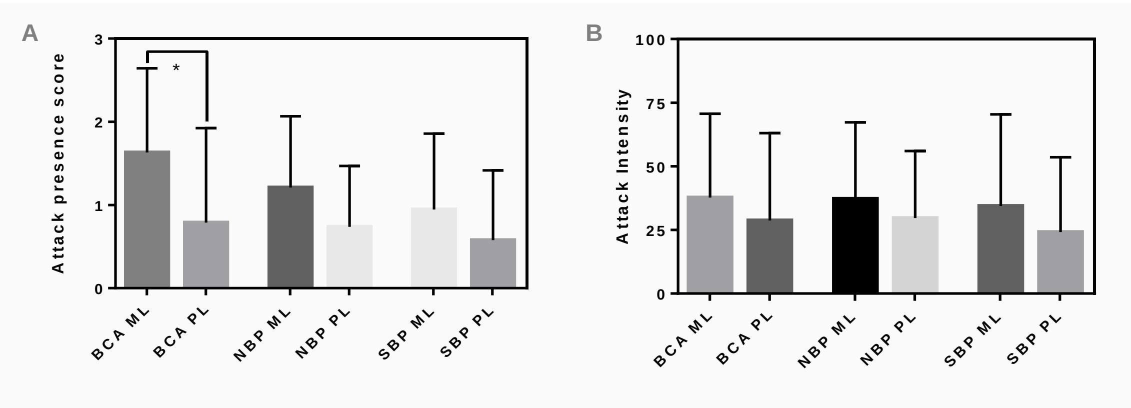


Figure 20. *Tetranychus urticae* Production Layer x Maintenance Intensity and Occupation comparison by treatment. Eggs treated with Biological Control Agent (predatory mites) releases, New Biopesticide (Cultaza SERV-MITE) and Standard Biopesticide (Sapec Boreal) were compared by layer both in Presence score [Panel A], using Mann-Whitney test, and Intensity rate [Panel B] using unpaired t test correlated using Pearson correlation coefficients from *T. urticae* effects on Presence score data during the first 10 weeks. Statistically differences ($p<0.05$) are noted with an '*'.

2.3. Harvest

Due to a low quality product, with high numbers of pest individuals, and poor appearance leading to a non-sellable product; commercial harvest did not occur.

Instead a pruning was performed, leading to a new restructuration process. This was done by ‘sacrificing’ and bending the best stems from the production layer, to recreate the maintenance layer using fresh and less injured material, attempting to restructure and improve the overall plant quality for a second harvest.

Remaining material with high levels of attack, and showing no possible recovery was pruned and removed from the greenhouse in order to achieve a reduction in pest numbers.

2.3.1. Quality and production

Since harvest did not took place, quality and production evaluation was not carried out.

3. Post pruning

3.1. Attack presence score and intensity rate evolution

Data sampling after the technical harvest was resumed. It was interesting to compare what the restructuration in ML and the new stems in the PL would score against previous data.

BCA (*P. persimilis* and *N. californicus*) would have new conditions for settling, and achieve a different level of control, in comparison with the data collected so far.

NBP treatment was dropped by the farmer in favour of the SBP, this was done by cost/effectiveness decisions.

3.1.1. *Maintenance Layer*

Mobile Forms

The two treatments had an inverse evolution, and statistically differences were found (P=0.079). None of treatments reach the score "3" (Average Class values: BCA = 0.8±0.4; SBP = 2.0±0). BCA treatment managed to drop the intensity to null values, giving good results in response to the restructuration the ML had.

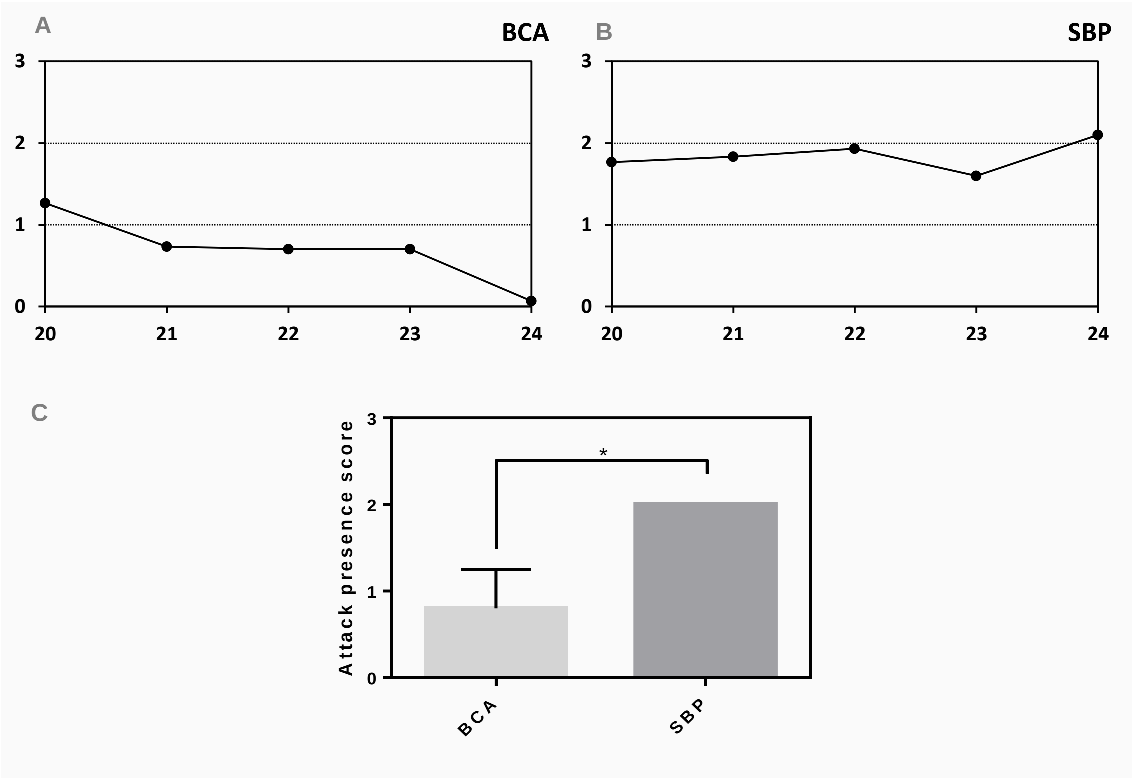


Figure 21. Mobile forms *Tetranychus urticae* individuals Presence Score evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effects on *T. urticae* Presence was scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

Intensity rate shows statistical difference between treatments (P=0.0064), with the number of attacked leaves dropping to near 0% using the predatory mites, while the SBP managed to stay over the 75% (Average Intensity rates values: BCA = 46.0±25.3 %; SBP = 89.3±6.7 %).

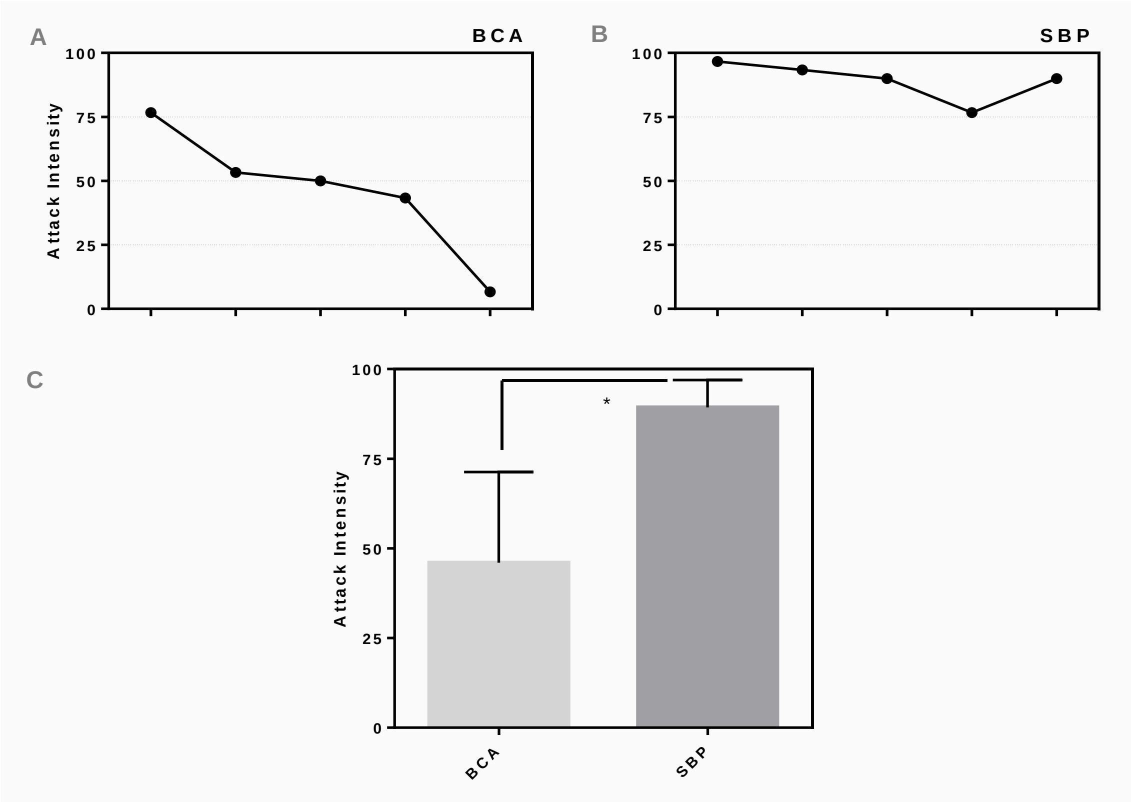


Figure 22. *Tetranychus urticae* mobile forms individuals Intensity rate evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effect on *T. urticae* ocupation rate were calculated from Presence data scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

Eggs

Egg presence score was reduced in BCA control method, as was expected with the reduced mobile forms numbers. SBP had also a small reduction, but still managed to finish above Score “2” (Average Score values: BCA = 0.4±0.5; SBP = 1.8±0.4). Difference was statistically meaningful (P=0.0238)

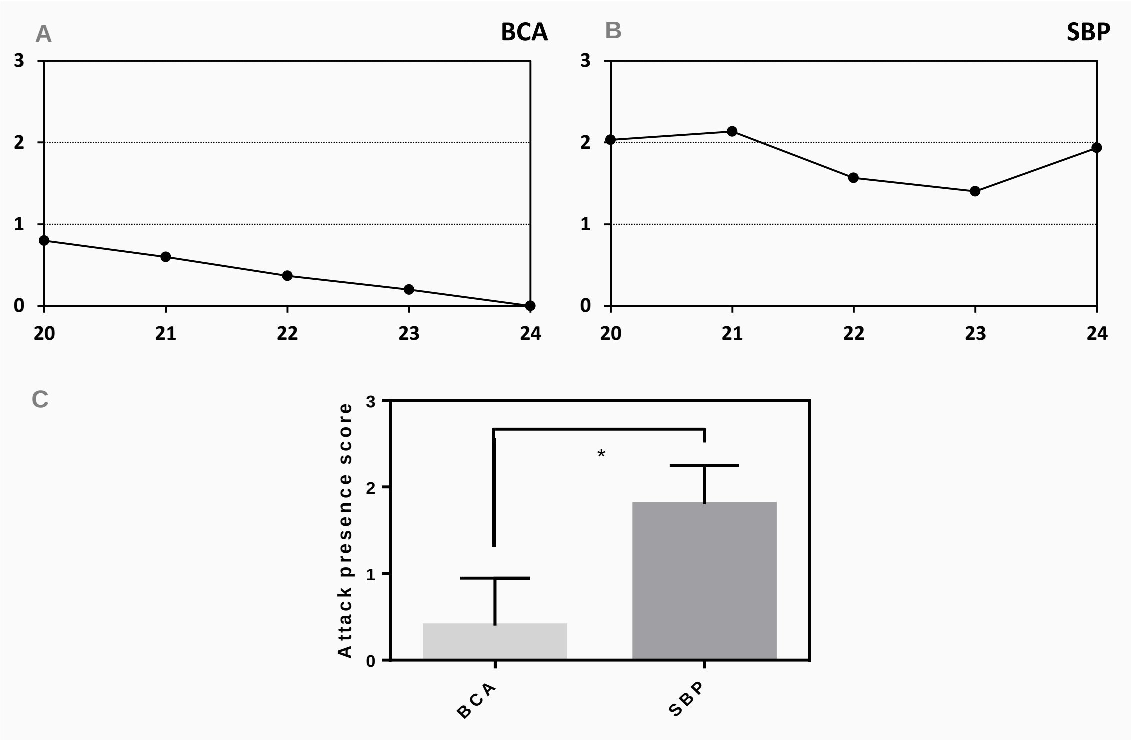


Figure 23. *Tetranychus Urticae* Eggs Presence Score evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effects on *T.urticae* Presence was scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

Attack Intensity in BCA shown positive, and almost overwhelming, results comparing with SBP (statistical difference, P<0.0001). SBP, although started with a low figure, the number of attacked leaves started to grow. This growth had a small decline by the end. (Average Intensity rate values: BCA = 4.7±4.5; SBP = 59.3±15.5).

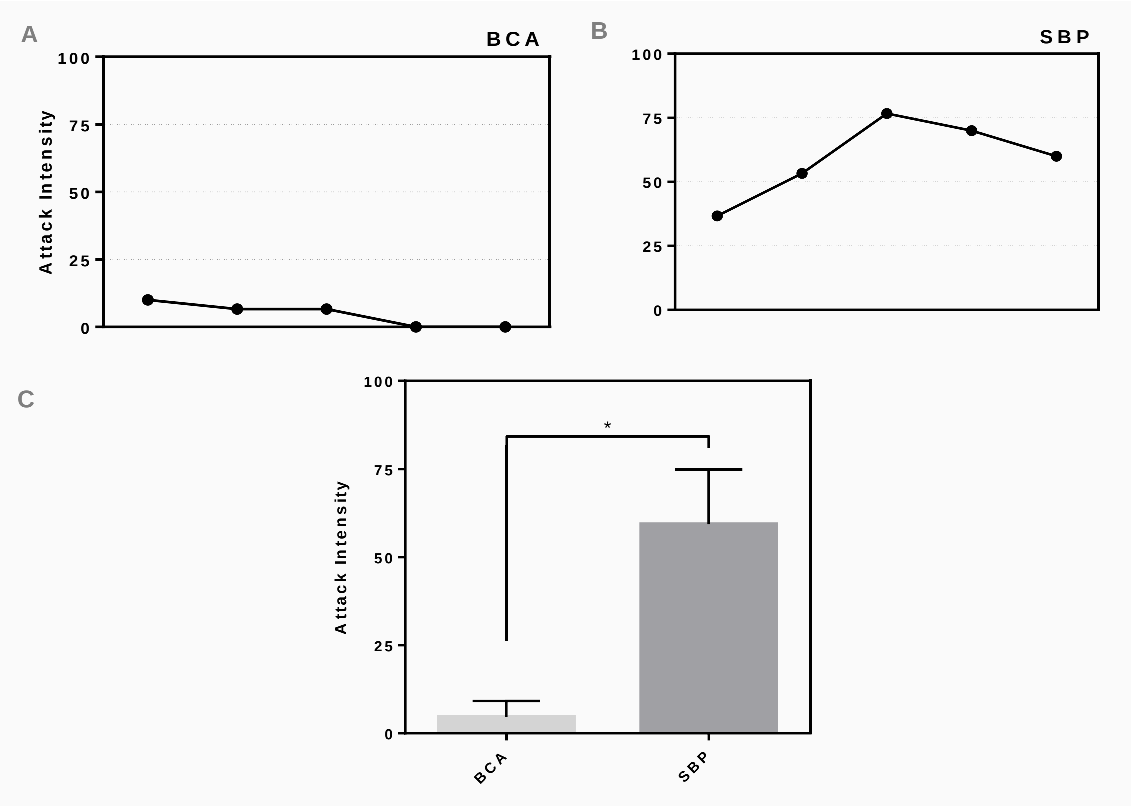


Figure 24. *Tetranychus urticae* egg Intensity rate evolution by treatment in maintenance layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effect on *T. urticae* ocupation rate were calculated from Presence data scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

3.1.2. Production Layer

Mobile Forms

BCA scoring numbers were inferior to the ones found in the SBP (P=0.0476), being able to achieve an overall 'null' score just before the harvest (Average score values: BCA = 0 ± 0; SBP = 1.0±0.7). Statistic difference was found.

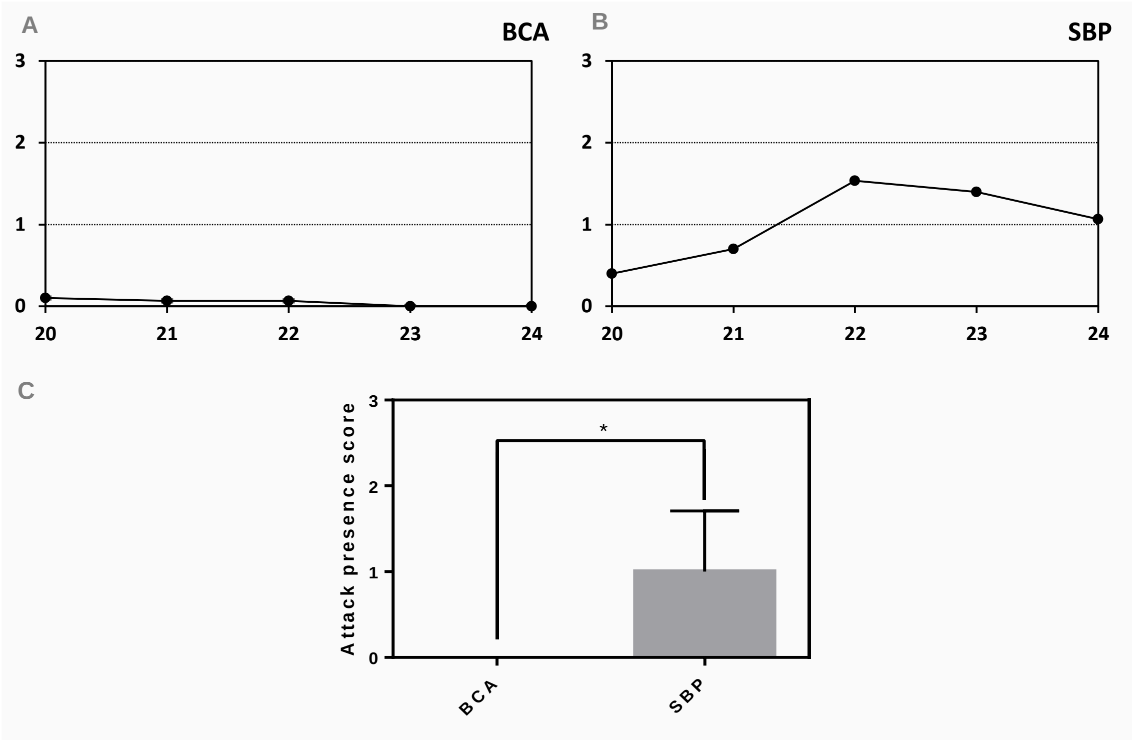


Figure 25. Mobile forms *Tetranychus urticae* individuals Presence Score evolution by treatment in production layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effects on *T. urticae* Presence was scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences ($p<0.05$) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

Number of attacked leaflets greatly reduced with BCA treatment in comparison with the SBP (P=0.0005), while, even the score was lower, the number of attacked leaves figured high values in the SBP (Average Score values: BCA = 29.3±21.8; SBP = 86.7±6.7)..

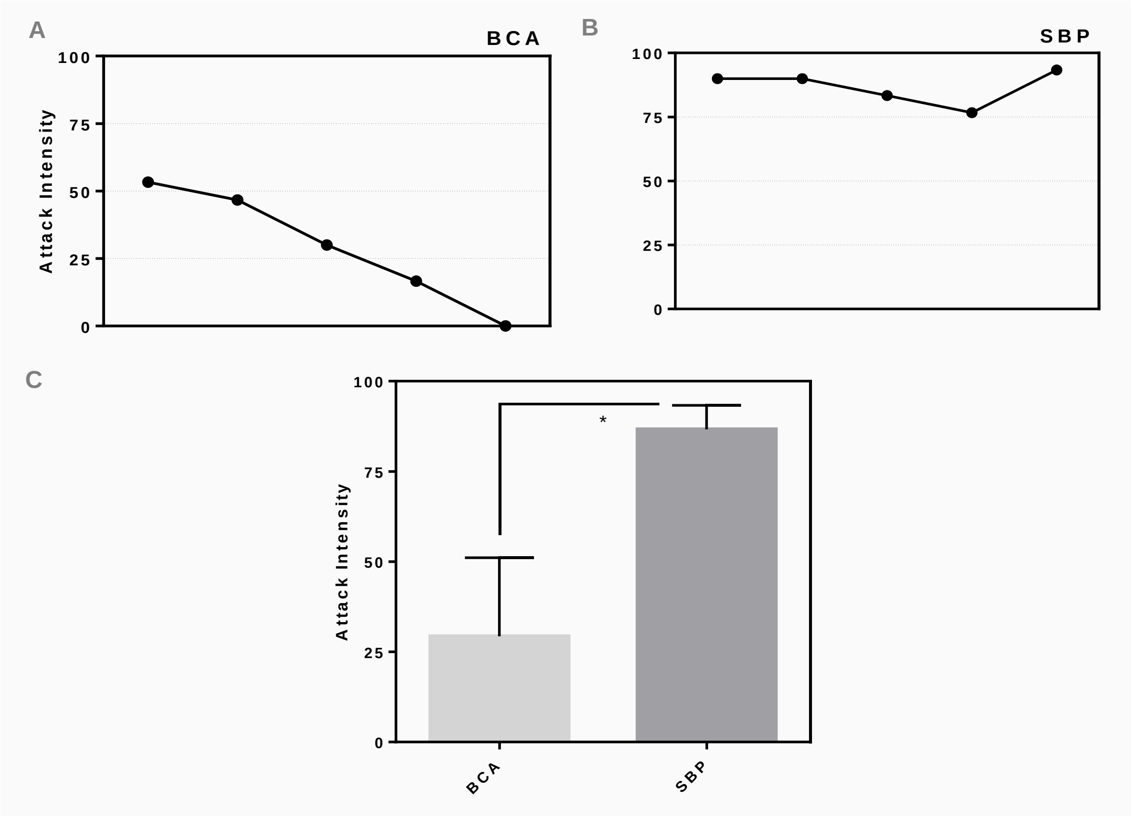


Figure 26. *Tetranychus urticae* mobile forms Instensity rate evolution by treatment in production layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effect on *T. urticae* ocupation rate were calculated from Presence data scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

Eggs

Although statistical difference was not found, a null presence score was maintained over till harvest in the BCA treatment, while SBP saw a small increase and ended up with a score of “1” (Average Score values: BCA = 0±0; SBP = 0.6±0.5).

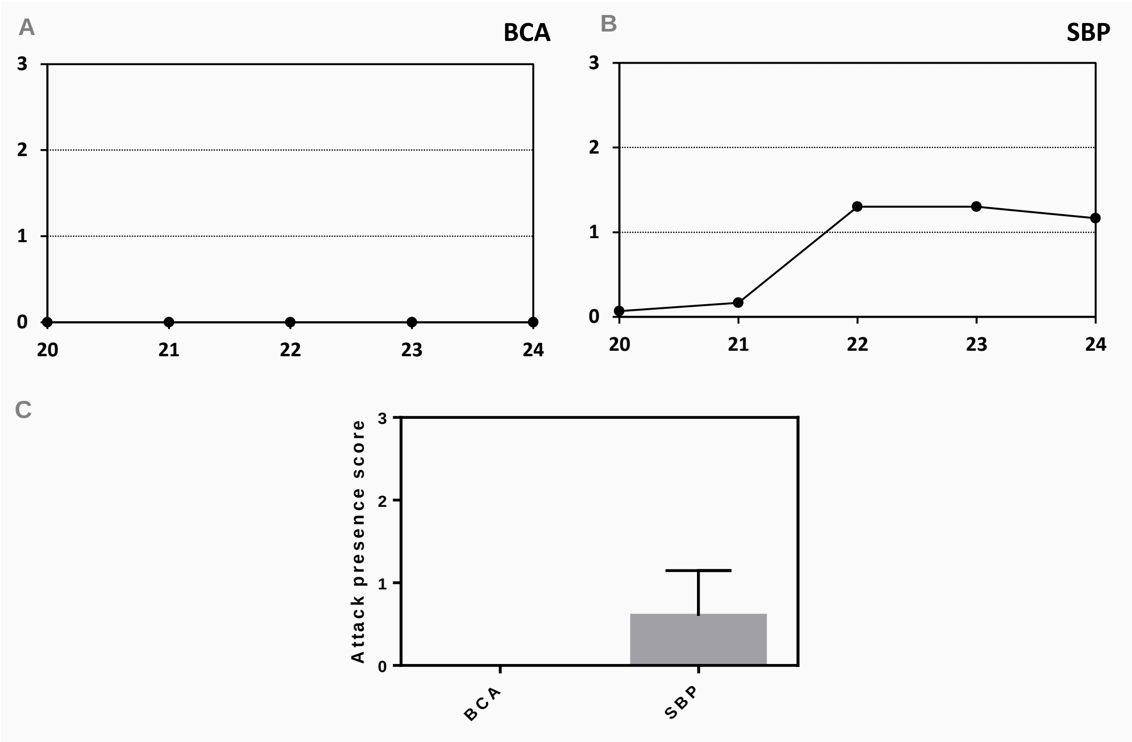


Figure 27. Tetranychus urticae Egg Presence Score evolution by treatment in production layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effects on *T. urticae* Presence was scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences ($p<0.05$) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

Intensity rate differences were accentuated and with statistic meaning (P=0.0111). BCA, as expected from the numbers above, had a null occupation, while numbers on SBP grew over time, with over 50% of the leaflets attacked at harvest (Occupation Average values: BCA = 0.0±0.0; SBP = 44.7±30.4).

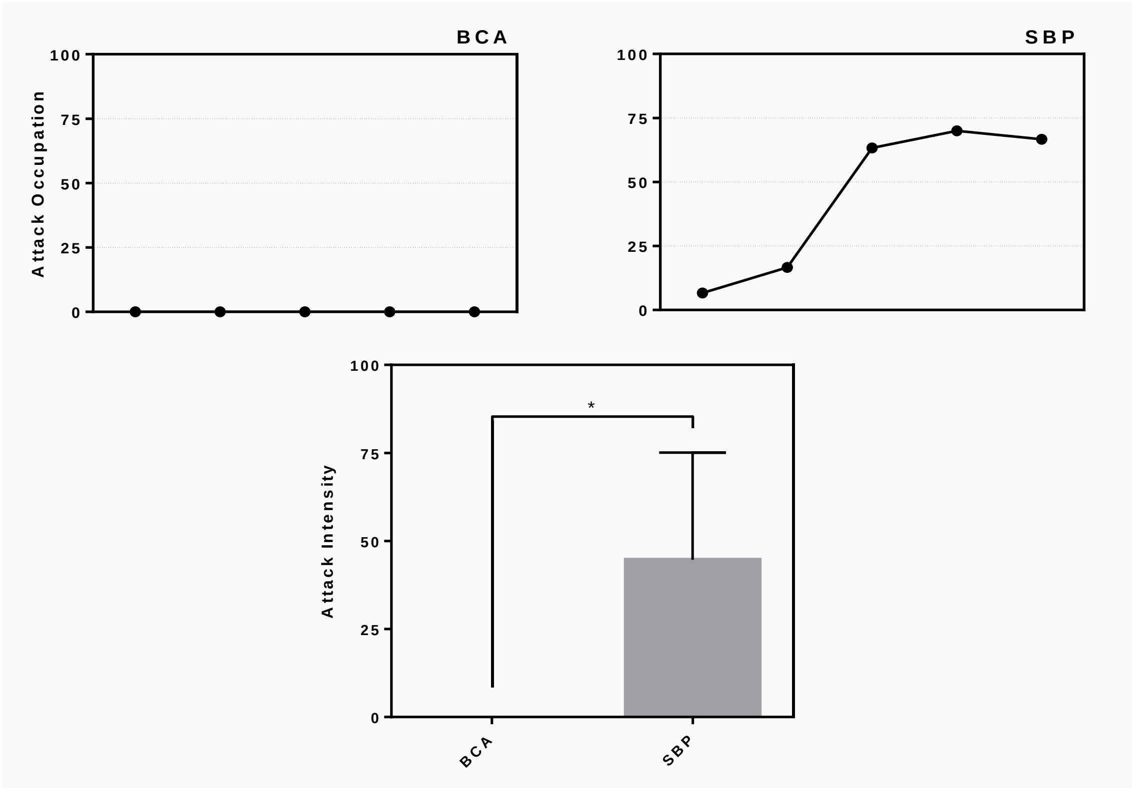


Figure 28. *Tetranychus urticae* egg Instensity rate evolution by treatment in production layer. Biological Control Agent (predatory mites) releases [panel A]; Standard Biopesticide (Sapec BOREAL) [panel B] effect on *T. urticae* ocupation rate were calculated from Presence data scored during the last 5 weeks prior to the end harvest. Results shown in these panels are mean observation value for all the readings during each sampling day. Panel C shows side-by-side comparison between average registered values. Statisticaly differences (p<0.05) are noted with a *, represented between which treatment a difference was found. Horizontal axis in panel A and B, represents Sampling Day.

3.2. Production Layer and Maintenance Layer relation

Attack intensity correlation [figure 29], was only found in the "SBP treatment, Eggs" (r=-0.4082, *P<0.0001*). Remaining treatments didn't show any correlation. This is explained with the low sampling number, both in weeks and attack absence.

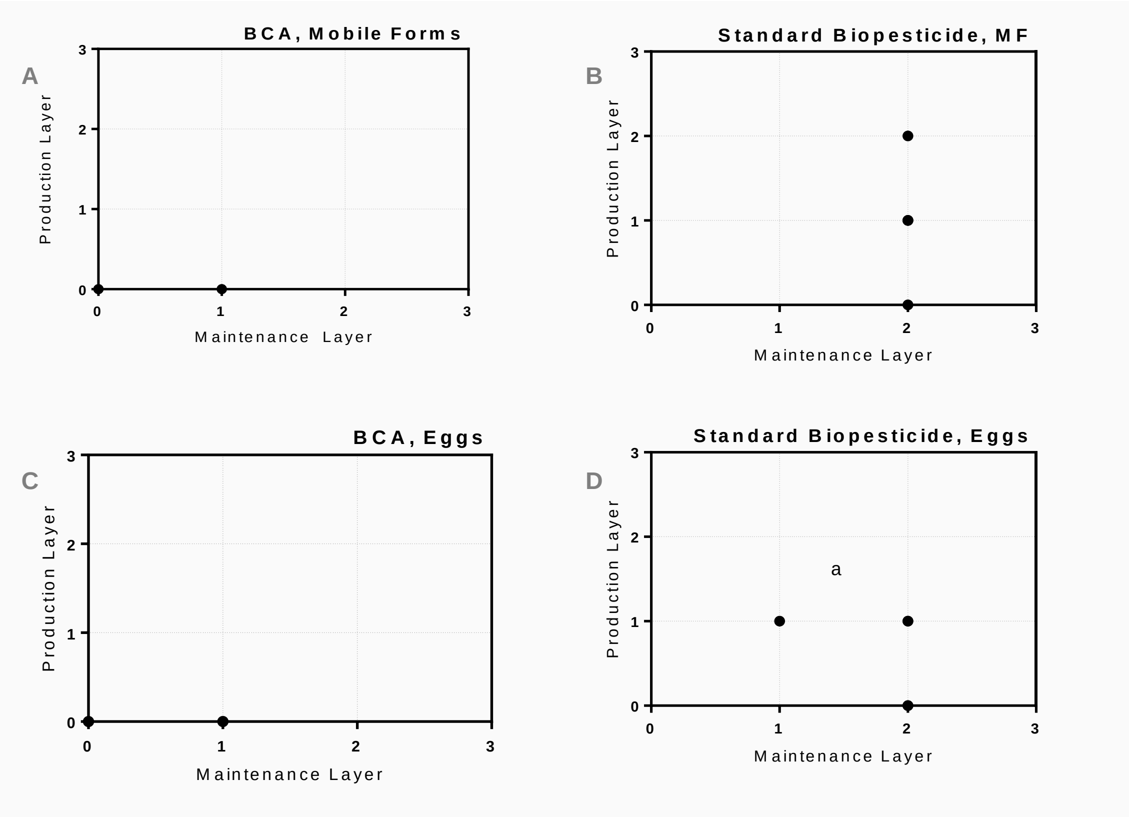


Figure 29. *Tetranychus urticae* Production Layer x Maintenance Layer Presence Score correlation by treatment. Mobile forms treated with Biological Control Agent (predatory mites) releases and Standard Biopesticide (Sapec Boreal) [respectively Panel A and B]; Egg treated with Biological Control Agent (predatory mites) releases and Standard Biopesticide (Sapec Boreal) [respectively Panel C and D] were correlated using Spearmen correlation from *T. urticae* effects on Intensity data during the last 5 weeks prior to the end harvest. Statistically differences ($p<0.05$) are noted with an 'a'.

Presence Score correlation results figured only statistical significance in the "BCA, Mobile forms" treatment, showing a high relation between both layer evolution (r=0.9308, P=0.0216). The remaining treatment sets showed no relation at all (Figure 29)

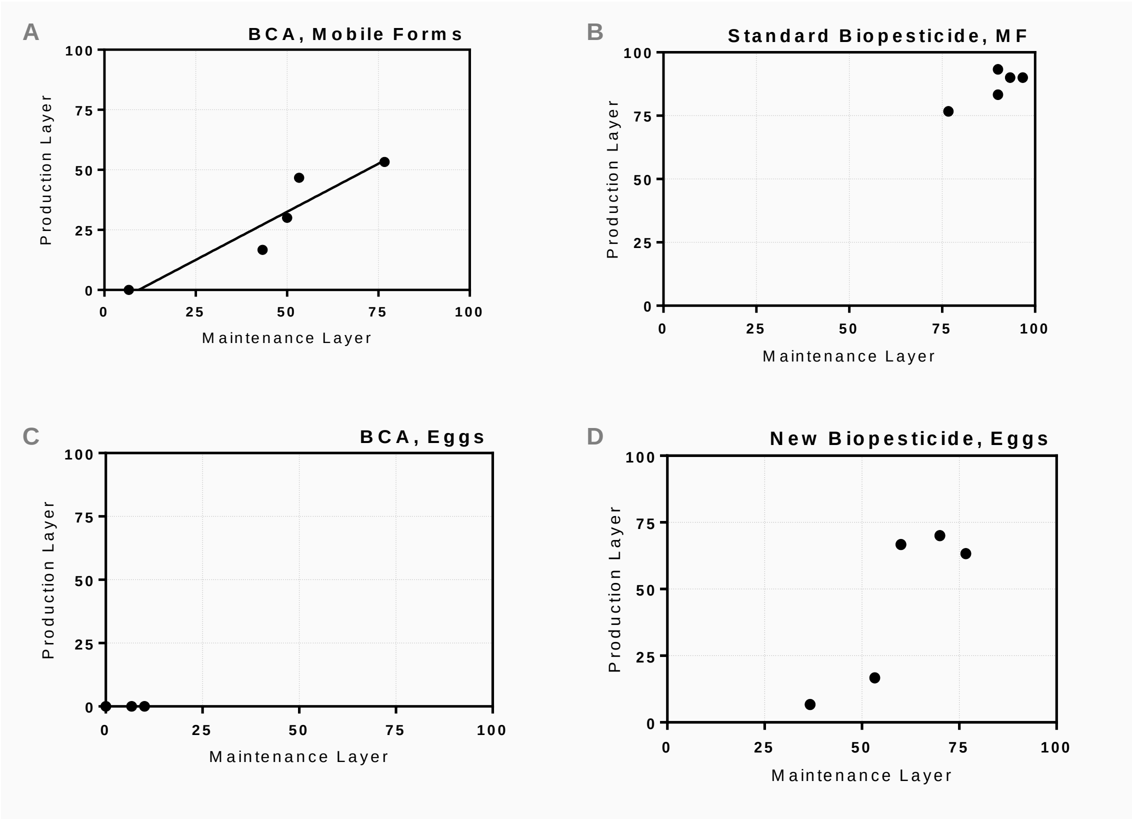


Figure 30. ***Tetranychus urticae*** **Production Layer x Maintenance Layer occupation rate correlation by treatment.** Mobile forms treated with Biological Control Agent (predatory mites) releases and Standard Biopesticide (Sapec Boreal) [respectively Panel A and B]; Egg treated with Biological Control Agent (predatory mites) releases and Standard Biopesticide (Sapec Boreal) [respectively Panel C and D] were correlated using Pearson correlation coefficients from *T. urticae* effects on occupation data during the last 5 weeks prior to the end harvest. Statistically differences (p<0.05) are noted with an 'a'.

Analysis

Mobile Forms

As before, the ML intensity was higher than the PL, being this difference statistical significant (both P=0.0476). Following the same pattern, attack-intensity was also higher in the ML, but the difference was not significant.

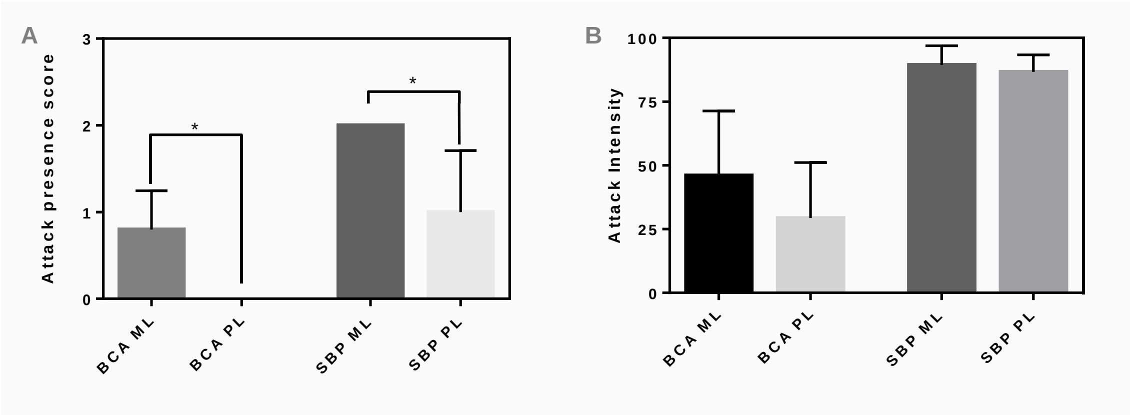


Figure 31. *Tetranychus urticae* Maintenance Layer x Production Presence Score and Intensity rate comparison by treatment. Mobile forms treated with Biological Control Agent (predatory mites) releases and Standard Biopesticide (Sapec Boreal) were compared by layer both in Intensity [Panel A], using Mann-Whitney test, and Occupation Panel B] using unpaired t test during the last 5 weeks prior to the end harvest. Statistically differences (p<0.05) are noted with a '*'.

Eggs

Both presence score and intensity rate were higher in the ML than the PL. Only in SBP presence score a significant difference was noticed (P =0.0317). Intensity wise only BCA treatment intensity rate was statistically significant (P=0.479).

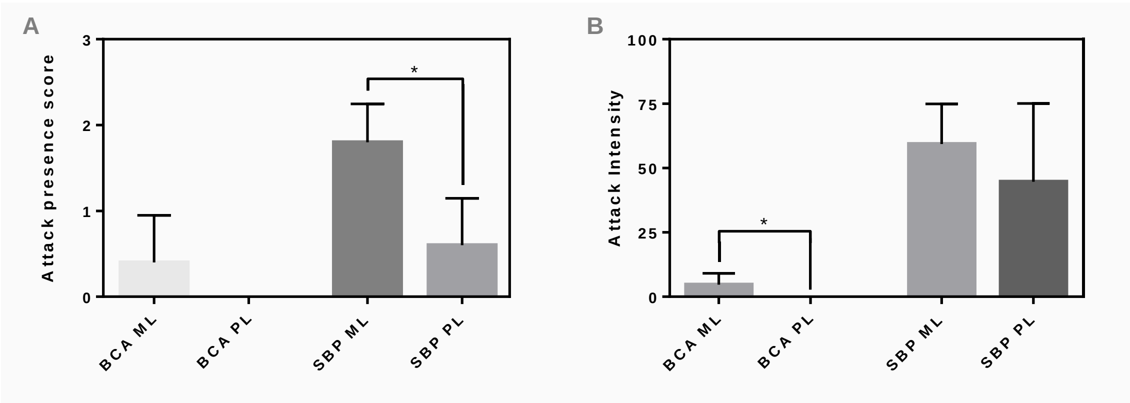


Figure 32. *Tetranychus urticae* eggs Production Layer x Maintenance Intensity and Occupation comparison by treatment. Eggs treated with Biological Control Agent (predatory mites) releases and Standard Biopesticide (Sapec Boreal) were compared by layer both in Intensity [Panel A], using Mann-Whitney test, and Occupation Panel B] using unpaired t test during the last 5 weeks prior to the end harvest. Statistically differences (p<0.05) are noted with a '*'.

4. Overview

4.1. Pre-Restructuration process

It’s easy to see that over both presence and intensity was similar, reaching high values in all treatments and both layers –with higher values in the Maintenance layer as expected. Marginally the BCA treatment gave the highest values and the SBP the lower ones.

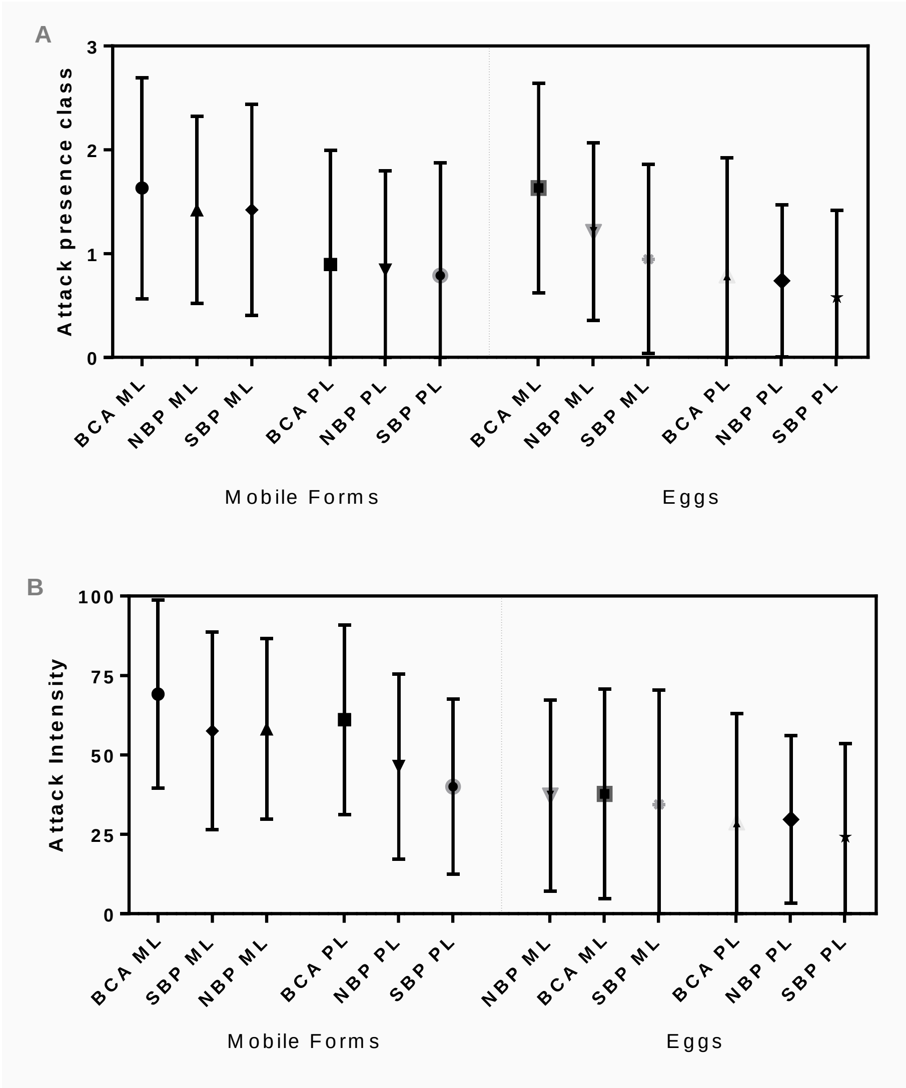


Figure 33. *Tetranychus urticae* Average Presence score and Intensity rate overview. Both Presence score [panel A] and Intensity rate [panel B] average values over the first 10 weeks are represented for all treatments.

4.2. Post Restructuration process

After the pruning and restructuration process, in a turn of events, BCA treatment, both in presence score and intensity rate had a drastic reduction, with acceptable numbers both in mobile forms and egg; SBP maintained high values.

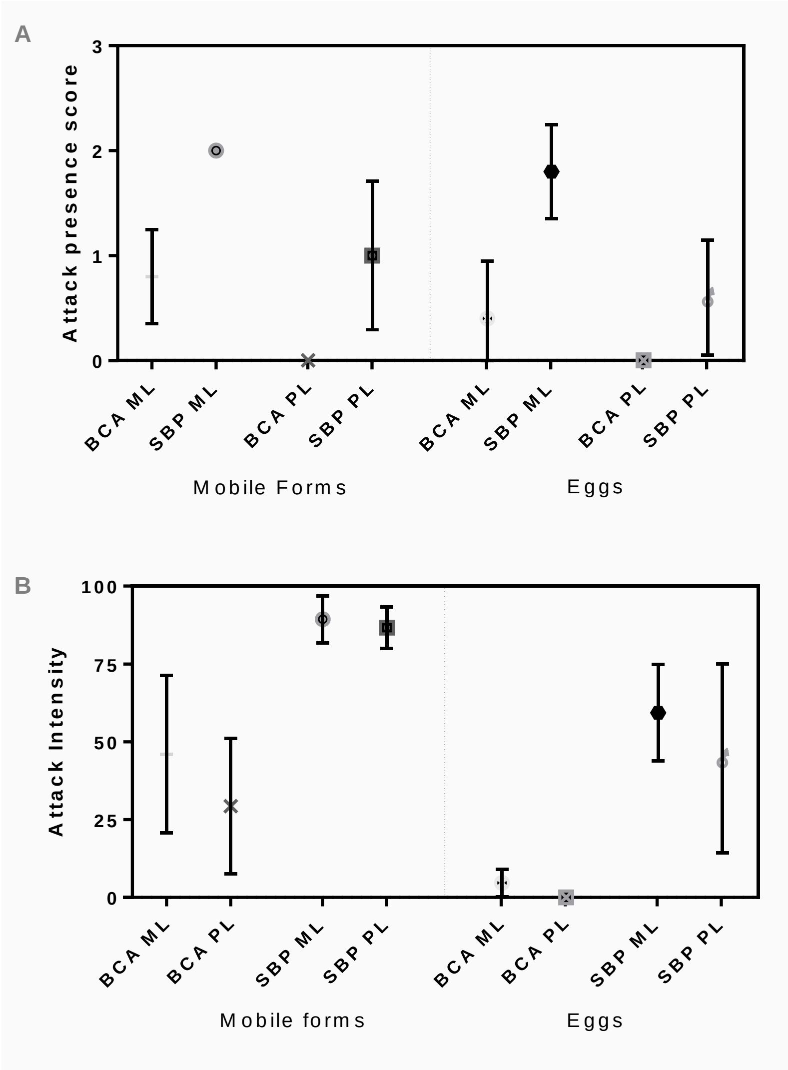


Figure 34. *Tetranychus urticae* Average Presence Score and Intensity rate overview Both presence score [panel A] and attack intensity [panel B] average values over the last 5 weeks prior to end for both BCA and SBP treatments.

4.3. Before and after

It was possible to review (Figure 33 and 34) that the maintenance process had took a positive effect, and differences were easily observed. Further comparison tests were done to strengthen this results.

4.3.1. Mobile forms

Side by side mean comparison in both presence score and intensity rate revealed a decrease in mobile forms numbers in both layers in BCA treatment, while SBP evidence the reverse situation.

Presence Score differences shows no statistical significance in any of the treatments, while in Attack occupation statistical differences are found in "BCA.PL" (P=0.0379) and both Layers in the "SBP" (P_{ML}=0.0357; P_{PL}=0.013).

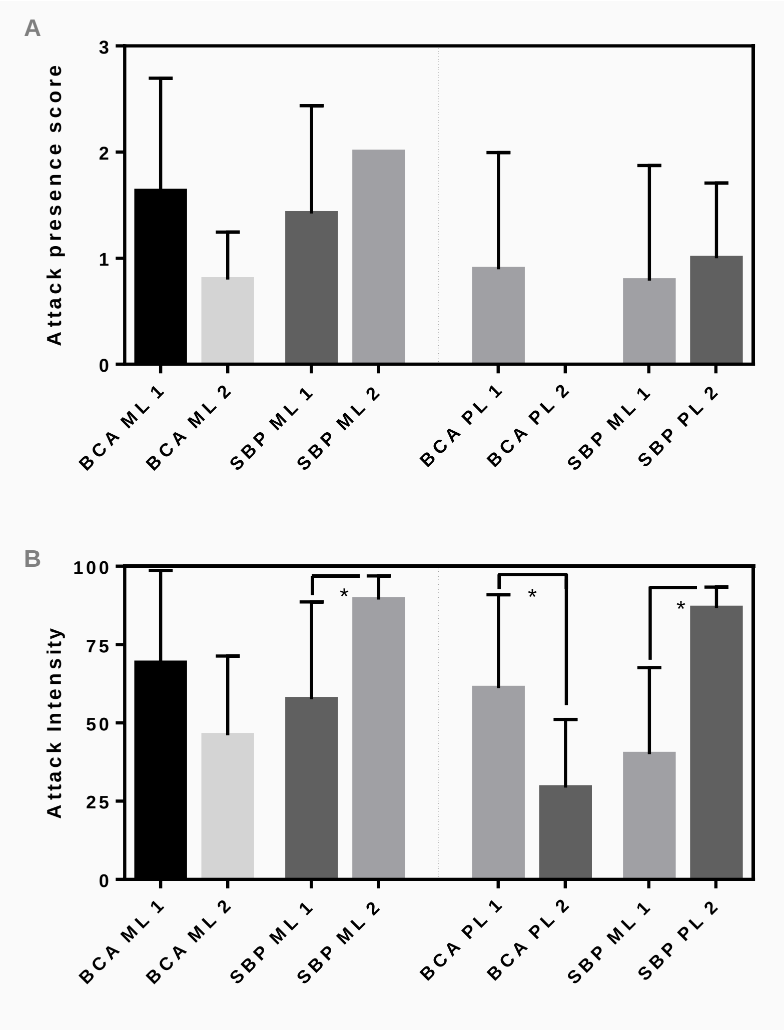


Figure 35. *Tetranychus urticae* mobile forms Presence Score and Intensity rate comparison between both periods (1: Before Technical Harvest; 2: After Technical Harvest) Both Intensity [panel A] and Occupation [panel B] average values are compared using Mann-Whitney test and unpaired t test (respectively) for BCA and SBP treatments.. Statistically differences (p<0.05) are noted with a '*'.

4.3.2. Eggs

Eggs follow the same patter as mobile forms, with both a reduction in the “BCA” treatment and increased numbers in “SBP”, except for the production layer intensity, which fell. Statistical meaning was only found in “BCA, ML” both in Presence score and Intensity ($P_{Presence}$=0.0163; $P_{Intensity}$=0.0386, respectively).

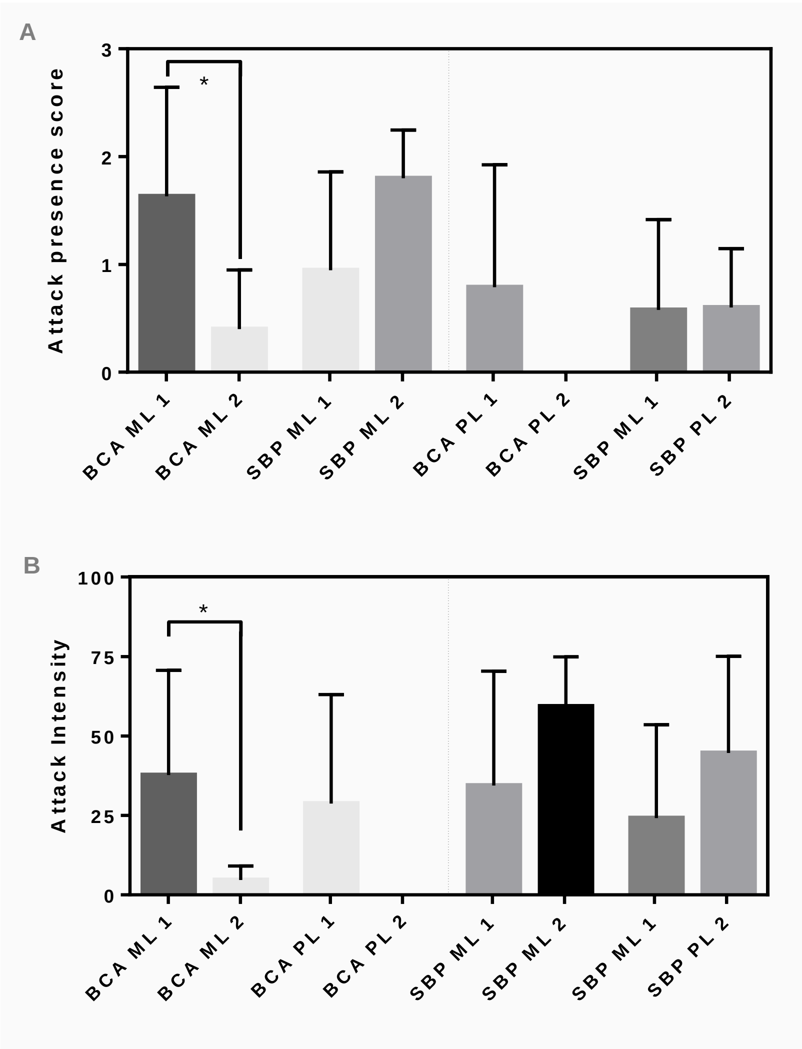
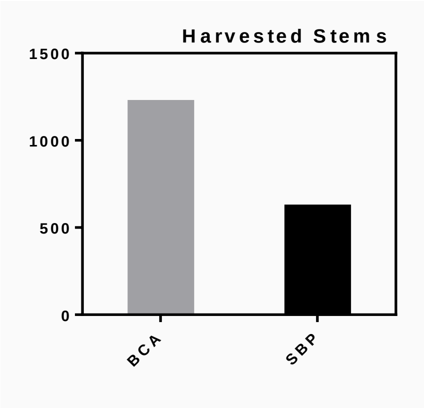


Figure 36. *Tetranychus urticae* egg Presence score and Intensity rate comparison between both periods (1: Before Technical Harvest; 2: After Technical Harvest) Both Intensity [panel A] and Occupation [panel B] average values are compared using Mann-Whitney test and unpaired t test (respectively) for BCA and SBP treatments.. Statistically differences (p<0.05) are noted with a ‘*’.

5. Productivity

Number of saleable/harvested stems can be observed in the Figure 36, with BCA having 1220 stems versus 620 stems in SBP treatment, nearly half the flowers in this treatment.



BCA	1220
SBP	620

Figure 37. Saleable stems obtained from end-harvest by treatment. Counting done by individual stem counting during the preparation and bunching process

6. Quality Analysis

As part of our quality analysis Stem Height (SH) and Flower Bud Width (FBW) were sampled and compared between BCA and SBP.

An average SH in was 51.61 ± 10.74 cm in BCA and 47.32 ± 9.80 cm in SBP.

FBW averaged 4.0 ± 0.8cm in BCA, and 3.7 ± 0.7cm in SBP .

Statistical meaning was found in both comparisons (P_{SH}=0.0095; P_{FBW}=0.0002).

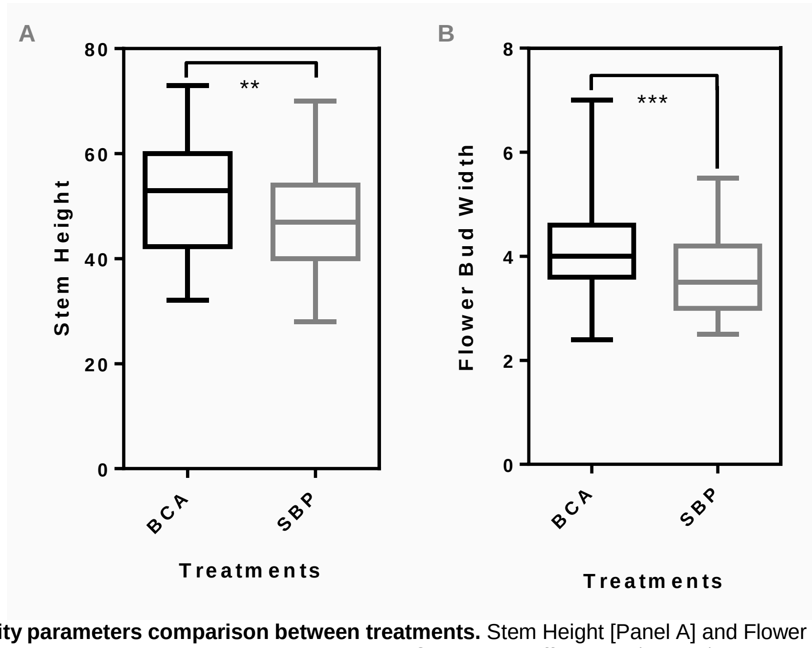


Figure 38. Quality parameters comparison between treatments. Stem Height [Panel A] and Flower Bud Width [Panel B] were compared between treatments using unpaired t-test. Statistically differences (p<0.05) are noted with a '*' (multiple * refers to a higher level of statistic differences).

6.1. End-product classification

End-product, “bunches”, type are determined by size categories. Table 15 represents the size distribution in percentage by each category by flowers of each trial treatment.

It is clear that most of bunches (39%) from flowers gathered with SBP treatment are from the lowest category, having low number of bunches from higher category (in its case not even reaching category 5). BCA shows a homogeneous results, and even having some bunches in a category 5.

Table 15 - Flower bunches size category distribution between treatments. Category is determined by the mean SH of the flowers using the the greenhouse workforce diision when packing the flower bunches.

Cat	≈ SH	BCA	SBP
1	35	*23%*	*39%*
2	45	*28%*	*29%*
3	50	*28%*	*19%*
4	55	*11%*	*13%*
5	60	*10%*	*0*

IV. Discussion

1. Initial sampling data

1.1. Pest evolution

High proliferation rate, usual of *T. urticae* [20.30], was observed, doubling their occupation in just five weeks. In an initial period, attack presence and Intensity was low, due to population's installation after the initial maintenance done in the crop. Soon their numbers grew, with higher presence scores and Intensity rate due to spreading into new shoots as expected [48].

1.1.1. Maintenance Layer

ML had a faster growing rate of scoring as initially the PL is almost non-existent, and *T. urticae* is able to feed of the growing leafs in this portion of the plant. Later they migrate into newer portions of the plant, searching for fresher leaves [48].

All treatments steady increased both in mobile forms and eggs, with none of them showing promising results in order to control the pest.

These growing numbers were accentuated in eggs found in the leaflets, mainly on the BCA treatment – this is because the pest control should be done mainly by the predatory mites, and as this control was failing. Eggs, which didn't had an effective control, were able to be laden; only prior to N. californicus released, and additional control was offer, since they pray on their eggs better than the *P. persimilis*. [17]. Unfortunately, control never reached acceptable levels.

1.1.2. Production Layer

As soon as the attack migrates into the PL, there was a massive bump in their presence score and intensity rate.

Possibly due to the new generations grown later in time, already gaining a number of resistance mechanisms as stated by [10], and even being more exposed to the treatments, they can endure them.

Overall numbers in ML were always superior, but attacks in both layers grew with some degree of relation, as expected since new generations tend to proliferate into new shoots.

Some high peaks followed by an accentuated fall in intensity is explained by treatment application of Floramite® SC, Nissorum® WP and Tenor® SC – done in all trial sets to aid controlling the main treatments. These however must be done with at least 2 weeks interval in order to refrain resistances build up.

1.2. Comparison between different control methods

1.2.1. Biological Control Agent

The BCA treatment was purposed as predatory mites have shown great results worldwide on greenhouses to control *T. urticae*, and have been one of the most successful methods [14,15,19]. This, however, was not the case in the first period of our essay, with BCA reaching a presence score of 3 and near 100% attack intensity.

We hypothesize this could be due to limitations during the initial BCA releases: the first release was over a month behind schedule due to logistic reasons, and failed due to the presence of an incompatible pesticide. This accelerated pest population growth, and even with occasional aiding treatments, numbers, especially in the ML were quite high – while numbers in the PL maintained a similar pattern with remaining treatments

In order to address this situation all the following releases after the second one saw the BCA density uprated from 10ind/m^2 to 16ind/m^2, in order to cope with the delay, by Koppert indication and with note that different ratios can strengthen control response [15].

As known [17], predatory mites releases should be as successful in controlling the pest as soon the predatory mites creates a well establish community, which was hampered by the already big and well establish *T. urticae* populations.

All sum up this lead to believe that the treatment could have good results with the right setup, which allowed it to continue after the maintenance harvest done.

1.2.2. New Biopesticide

The NBP trial, using **Cultaza's** *Serv-Mite* had results between the use of the BCA and the Standard treatment, which saw high pest population and occupation rate. Serv-Mite acts by contact, disabling breathing, egg-laying and feeding, but, as results didn't meet the expectations, high dosage, and previous attempts on using this treatment had probable lead to resistance [10].

The cost and the logistic involved to maintain this treatment lead to the farmer discontinuing its use and switch the trial into the standard one after the pruning and restructuration process done.

1.2.3. Standard Biopesticide

By the end of the first sampling period had started to show signs of regression in both presence and intensity rate, especially on the Production layer. This regression must have been the result of the introduction of new products in aiding the control such as the Silwet Gold®, and the intercalated use of Floramite® SC and Nissorum® WP, which had a lower resistance since their use was less regular than the weekly Boreal® [45].

2. Technical Harvest / Restructuration process

Schedule for the end of the tenth week, the first harvest didn't take place. The low amount of saleable stems with low quality flowers (figure 37-38), and high number of mites, easily seen on clear sight were the main reason; this however made the farmer take the decision to do a restructuration and a maintenance pruning all-round [24].

The majority of attacked shoots from the maintenance layer were hand-removed and new shoots from the PL, with less mite numbers, were bent to rebuild the ML.

This process was done in order to find a solution to the crop before taking any more drastic measures, such as complete pruning of the plants or even removing the whole variety.

2.1. Pest evolution after restructuration

As results until the restructuration process were in general poor, with none significant result whatsoever, to improve the crop situation and offer a good answer to the farmer, a follow up on the two remaining trials were done until a successful harvest could be made

As soon as the first sampling was done presence had already decreased, although intensity rates was still high - expected since there was still infected material in the plants.

In a turn of events, BCA was now the treatment with best results, and managed those numbers throughout the trial, until harvest. This corroborates all data gathered about the need for the BCA, especially predatory mites, to establish a good population so they can thrive and overcome the pest mite [13].

ML achieved a great reduction in both figures using the BCA treatment.

In SBP treatment, although managed to reduce their presence, both in mobile forms and eggs, to medium levels, was not so successful as the use of BCA, and the difference was significant.

New shoots in the productivity area were, in both cases, healthier looking with lesser numbers of *T. urticae* on them, but only BCA managed to contain acceptable levels.

2.2. Treatments overview

The use of auxiliary predatory mites (BCA) were able to greatly reduce not only the pest presence to been able to reduce the intensity of the attacks to a bare minimum, with both scoring and intensity sitting in values near 0.

SBP, attained better results during this second period, although the less positive pest evolution could point the better results to the cultural control done at the end of the 10th week, which managed a reduction of infected material and optimization of the contact area, since the aforementioned point aided in creating better circulation and openings in the canopy, which improved the treatments efficacy [17,24]

Unfortunately, at the very end of the essay, there was a slightly rise in both score and intensity. Overall, less satisfying results could be explained by the natural, highly developed, *T. urticae*. resistance mechanism [10].

3. Harvest results

The final comparison to be done was the post-harvest data gathered from both production numbers and quality measurements (Stem Height and Flower Bud Width).

3.1. Quality

Not surprising BCA surpassed both in SH and FBW. These higher numbers represent stronger, taller flowers with a good upright posture, consistent, good looking flower buds, clean of mites and other insects.

While in the SBP many flowers shown a degree of curvature along other appearance problems which lead to most of them to be discarded. Many non-saleable characteristics were present – such as torn and burnt petals, chlorotic leaves, thin and fragile stems.

Although the result seems positive for the BCA treatment, it matters to notice that there is space for improvement since mean SH reported by the grower (Schreurs, NL), is between 60 cm to 90 cm. FBW is reported to be between 9 cm and 11 cm, but it's not stated how nor when these measurement is made, which can greatly differ due to bud opening.

3.2. Production

A difference was observed from the stems obtained from each treatment, with BCA (1220 saleable stems) doubling SBP numbers (620 saleable stems). This difference was stated well before the harvest, as many of the flowers shown bad quality characteristics, such as short stems and severe attacks from the *T. urticae*, which ended being discarded.

Differences between flowers from both trials were reflected not only in numbers, but in quality factors too. Flowers in BCA treatment were, on average, more than 4cm longer than flowers in SBP treatment.

In addition, as seen on table 15, BCA flowers SH distribution tended towards higher numbers, which enabled an additional category, with an average SH of 60 cm^2 .

V. Conclusions

1. End-Analysis

Plants affected with *T. urticae* needs to be promptly addressed at risk of large quality and productivity losses. As an ornamental culture, cut roses, needs a higher degree of attention due to end-market value is given by its appearance characteristics, on which pest's attacks could play a crucial role.

Classic and long-time used treatments, such as Abamectine, while still oferring some degree of protection, carry an over-time effectiveness loss. This is often exacerbated in greenhouse environment as isolated population's growth strengthen the natural *T. urticae* resistance mechanisms.

An industry of new bio pesticides, using natural products and/or different action methods are growing and delivering promising products. Nevertheless results obtained in this work with Cultaza SERV-MITE were not good, demonstrating it incapable of handling the *T. urticae,* and turned out to be discarded for its low cost/effectiveness ratio.

As a good example of a sustainable and integrated pest management, the use of a Biological Control Agent, such as a predatory mite, was found to be the the overall best way of handle this pest. Although this was not the case from the beginning, as initial non optimal conditions may have hampered its effectiveness. So a base BCA population should be advisable in order to sustain the pest at minimal levels from the start; aditionaly, means should be taken in order to maintain the BCA population, which tipicaly reduces with lower pest numbers, as there is less food and limits their growth.

Lastly, cultural control methods, are a great way of managing possible high level of attacks. Possibly the best way to reduce inoculum is the manual removal of affected material.

2. User-friendly methodology overview and conclusions

The knowledge of a strong positive correlation outcome between maintenance layer and production layer assigned level, and the fact of maintenance layer attack is always slightly higher than the production layer, allow us to further suggest to restrict observations solely to maintenance layer without any increased risk.

The finding that the risk due to mobile forms is similar to the risk due to eggs also suggests no need to sample egg numbers – unless further information is need for decision making.

Moreover, with the gathered readings of over 8000 leaflets in the course of 15 weeks, we were able to recommend and Economic threshold that is both safe – as not being near an extreme point, as initialy was; and easly obtainable using this methodology. Plants scoring level “2” were so heavily attacked and on the edge of being considered lost plants, as such an economic threshold score of “1”, based on scoring table of the purposed methodology is suggested, discarding the previous score of “3”, as the highest.

3. Study impact

At the end of our essay, the farmer made the decision to fully convert the whole greenhouse into the use of BCA as the main treatment at light of the good results obtained on the course of our work.

The choice of application was Koopert’s SPICAL-PLUS, packing a set of *N. californicus* in a paper sachet with a hook. This method offers a balanced release, has it removes the need for the farmer to do the direct application from the “bottle”, reducing the less homogeneous application and protection. This method also allows an extended period of protection as releases of the predatory mite are done over the course of 4 weeks with no intervention needed. This answered a long lasting problem, leaving expectations that future harvest could improve both in quality and numbers. This helps redirecting costs to facility and production upgrades and new investments, increasing market value and integration.

Lastly, the easy to use Risk Assessment method shown proof of validation and should be ready to be used in any commercial greenhouse rose production. Further use in different set-ups are a good way of strengthen and continuously validate the method.

4. Future Research

At light of this study new challenges have risen. During the selection of the variety to receive the trials, while “White-Naomi” showed better logistic characteristics, it was too stated by the farmer that these variety seemed to be the most susceptible to *T. urticae* attacks, showing a decay in quality and numbers at a faster pace than all the other varieties grown at the same greenhouse. It is suggested that a set of a Molecular and Cellular Biology trials be done in order to address these theory.

While cost numbers were mainly dealt by the farmer, it would be interesting to realize an Economical analysis towards the costs in the treatment methodology taken, with both analysis on treatment cost but also on obtained market value.

Finally a follow up of the current work could be done in order to assure maintenance on results provided by these trials: “Was the BCA treatment truly successful?” or “Was the treatment continued?” Agronomics is not static, and maintenance is needed in order to achieve good results during the course of the exploration.

VI. References

1. Defra. Basic Horticultural Statistics 2013. *Dep. Environ. food Rural Aff.* 23 (2013).
2. CBI. *CBI Product Factsheet : Fresh Cut Roses in the European Market*. *CBI Market Intelligence* (2015).
3. INE. *Floricultura e Plantas Ornamentais 2012*. (2013).
4. Ehret, D. L., Alsanius, B., Wohanka, W., Menzies, J. G. & Utkhede, R. Disinfestation of recirculating nutrient solutions in greenhouse horticulture. *Agronomie* **21.** 323–339 (2001).
5. Sonneveld, C. Effects of salinity on substrate grown vegetables and ornamentals in greenhouse horticulture. 149 (204AD).
6. Samartzidis, C., Awada, T., Maloupa, E., Radoglou, K. & Constantinidou, H. I. a. Rose productivity and physiological responses to different substrates for soil-less culture. *Sci. Hortic. (Amsterdam).* **106,** 203–212 (2005).
7. Hossain, S., Haque, M. M. & Naher, N. Control of two – spotted spider mite Tetranychus urticae Koch (Acari : Tetranychidae) by some selected chemicals. **25,** 15–18 (2006).
8. Nicetic, O., Watson, D. M., Beattie, G. a C., Meats, a. & Zheng, J. Integrated pest management of two-spotted mite Tetranychus urticae on greenhouse roses using petroleum spray oil and the predatory mite Phytoseiulus persimilis. *Exp. Appl. Acarol.* **25,** 37–53 (2001).
9. Landeros, J., Chávez, E. C., Aguirre, L. A., Canales, R. F. & Fuentes, Y. M. O. Demographic Parameters of Tetranychus urticae (Acari : Tetranychidae) on Four Rosa sp . Cultivars. **96,** 1508–1512 (2013).
10. Khajehali, J., Van Nieuwenhuyse, P., Demaeght, P., Tirry, L. & Van Leeuwen, T. Acaricide resistance and resistance mechanisms in Tetranychus urticae populations from rose greenhouses in the Netherlands. *Pest Manag. Sci.* **67,** 1424–1433 (2011).
11. Herron, G., Edge, V. & Rophail, J. Clofentezine and hexythiazox resistance in Tetranychus urticae Koch in Australia. *Exp. Appl. Acarol.* **17,** 433–440 (1993).
12. Kim, S. & Yoo, S. Comparative toxicity of some acaricides to the predatory mite, Phytoseiulus persimilis and the twospotted spider mite, Tetranychus urticae. *BioControl* 563–573 (2002). at <http://link.springer.com/article/10.1023/A:1016585607728>
13. Forero, G., Rodríguez, M., Cantor, F., Rodríguez, D. & Cure, R. Criterios para el manejo de Tetranychus urticae Koch (Acari : Tetranychidae) con el ácaro depredador Amblyseius (Neoseiulus) sp . (Acari : Phytoseiidae) en cultivos de rosas Criteria for the management of Tetranychus Urticae Koch (Acari : Tetranych. **26,** 78–86 (2008).
14. Souza-pimentel, G. C. De *et al.* Biological control of Tetranychus urticae (Tetranychidae) on rosebushes using Neoseiulus californicus (Phytoseiidae) and agrochemical selectivity. **40.** 80–84 (2014).
15. Greco, N. M., Sánchez, N. E. & Liljesthröm, G. G. Neoseiulus californicus (Acari: Phytoseiidae) as a potential control agent of Tetranychus urticae (Acari: Tetranychidae): Effect of pest/predator ratio on pest abundance on strawberry. *Exp. Appl. Acarol.* **37,** 57–66 (2005).
16. Easterbrook, M. a., Fitzgerald, J. D. & Solomon, M. G. Biological control of strawberry tarsonemid mite Phytonemus pallidus and two-spotted spider mite Tetranychus urticae on strawberry in the UK using species of Neoseiulus (Amblyseius) (Acari: Phytoseiidae). *Exp. Appl. Acarol.* **25,** 25–36 (2001).
17. Forero-Patiño, J. J., Argüelles, Z. P., Cantor, R. F. & Rodríguez, C. D. Bioensayo para evaluar la compatibilidad de adultos de Neoseiulus californicus (Parasitiformes : Phytoseiidae) con algunos pesticidas de uso comercial en condiciones semicontroladas. *Agron. Colomb.* **28,** 273–280 (2010).
18. Hilarión, A., Niño, A., Cantor, F., Rodríguez, D. & Cure, J. R. Criterios para la liberación de Phytoseiulus persimilis Athias Henriot (Parasitiformes:

Phytoseiidae) en cultivo de rosa. *Agron. Colomb.* **26,** 68–77 (2008).
19. Skirvin, D. J. & Fenlon, J. S. Plant species modifies the functional response of Phytoseiulus persimilis (Acari: Phytoseiidae) to Tetranychus urticae (Acari: Tetranychidae): implications for biological control. *Bull. Entomol. Res.* **91.** 61–67 (2001).
20. Gorman, K., Hewitt, F., Denholm, I. & Devine, G. J. New developments in insecticide resistance in the glasshouse whitefly (Trialeurodes vaporariorum) and the two-spotted spider mite (Tetranychus urticae) in the UK. *Pest Manag. Sci.* **58,** 123–130 (2002).
21. Abdullah, M. G. *et al.* Rose. *Encyclopædia Britannica Inc.* (2015). at <http://www.britannica.com/plant/rose-plant>. Access date: 2015-07-01
22. Chapman, L., Drage, N. & Durston, D. *Tea Roses: Old Roses for Warm Gardens*. (Rosenberg Publisher, 2008). at <https://books.google.pt/books?id=a2_g1faKWdYC>
23. Barrett, J. *Yes, You Can Grow Roses*. (Texas A&M University Press, 2013). at <https://books.google.pt/books?id=rfpJAAAAQBAJ>
24. Maden, E. van der *et al. Handbook for Greenhouse Rose Production Ethiopia*. (Wageningen UR, 2011).
25. Ministério da Agricultura, do desenvolvimento R. e das P. Flores. *Gab. Planeam. e Polit.* (2007).
26. VANOS, E. Closed soilless growing systems: A sustainable solution for Dutch greenhouse horticulture. *Water Sci. Technol.* **39,** 105–112 (1999).
27. Castilla, N. *Greenhouse Technology and Management*. (CABI, 2013). at <https://books.google.pt/books?id=PcKDwgaHhaMC>
28. Ehret, D. L., Menzies, J. G. & Helmer, T. Production and quality of greenhouse roses in recirculating nutrient systems. *Sci. Hortic. (Amsterdam).* **106,** 103–113 (2005).
29. Pardossi, a *et al.* Fertigation and Substrate Management in Closed Soilless Culture. (2011).
30. Chow, A., Chau, A. & Heinz, K. M. Reducing fertilization for cut roses: effect on crop productivity and twospotted spider mite abundance, distribution, and management. *J. Econ. Entomol.* **102,** 1896–1907 (2009).
31. Dixon, M. a., Butt, J. a., Murr, D. P. & Tsujita, M. J. Water relations of cut greenhouse roses: The relationships between stem water potential, hydraulic conductance and cavitation. *Sci. Hortic. (Amsterdam).* **36,** 109–118 (1988).
32. Cabrera, R. I., Evans, R. Y. & Paul, J. L. Cyclic nitrogen uptake by greenhouse roses. *Sci. Hortic. (Amsterdam).* **63,** 57–66 (1995).
33. Albajes, R., Lodovica Gullino, M., van Lenteren, J. C. & Elad, Y. *Integrated Pest and Disease Management in Greenhouse Crops*. *Developments in Plant Pathology* **14,** (Kluwer Academic Publishers, 1999).
34. Joshel, C. & Melnicoe, R. Crop Timeline for California Greenhouse Grown Cut Roses. (2004).
35. Chandler, S. F. & Lu, C.-Y. Biotechnology in ornamental horticulture. *Vitr. Cell. Dev. Biol. - Plant* **41.** 591–601 (2005).
36. Boller, E. F. *et al.* Integrated production: principles and technical guidelines. *Bull. OILB/SROP* **27,** 1–12 (2004).
37. Daughtrey, M. L. & Benson, D. M. Principles of plant health management for ornamental plants. *Annu. Rev. Phytopathol.* **43,** 141–169 (2005).
38. Riley, D. G. Economic Injury level and Economic Threshold concepts in pest management. *Am. Entomol.*
39. Meyer, J. r. Economic Injury Level. (2003). at <http://www.cals.ncsu.edu/course/ent425/tutorial/economics.html>
40. Hoover, G. A., Moorman, G. W., Richards, K. M. & Gripp, S. I. *Woody Ornamental Insect, Mite, and Disease Management*. (Pennsylvania State University, 2011).

41. Ec. *Flowers and Ornamental Plants Statistics*. (2013).
42. Saxena, M. *Hand Book on Horticulture Statistics*. (Government of India, 2014).
43. Spray, R. Periodic Price Information : Floriculture Products - European Markets FloraHolland ' s First Half-Year Accumulated Data , Weeks 1 through 26 2013. (2013).
44. MPS & International Trade Center. *MPS-ABC*. (2015).
45. Vassiliou, V. A. & Kitsis, P. Acaricide Resistance in Tetranychus urticae (Acari : Tetranychidae) Populations from Cyprus Acaricide Resistance in Tetranychus urticae (Acari : Tetranychidae). **106,** 1848–1854 (2012).
46. Opit, G. P., Nechols, J. R. & Margolies, D. C. Biological control of twospotted spider mites, Tetranychus urticae Koch (Acari: Tetranychidae), using Phytoseiulus persimilis Athias-Henriot (Acari: Phytoseidae) on ivy geranium: Assessment of predator release ratios. *Biol. Control* **29,** 445–452 (2004).
47. Cultaza. *Controlo de Aranhiço Vermelho em Roseiras*. (2012).
48. Fasulo, T. R. & Denmark, H. a. Twospotted Spider Mite , Tetranychus urticae Koch (Arachnida : Acari : Tetranychidae) 1. 1–5 (2012).
49. Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W. & Tirry, L. Acaricide resistance mechanisms in the two-spotted spider mite Tetranychus urticae and other important Acari: A review. *Insect Biochem. Mol. Biol.* **40.** 563–572 (2010).
50. Scopes, N. E. a. Mass-Rearing of Phytoseiulus riegeli Dosse for Use in Commercial Horticulture. *Plant Pathol.* **17,** 123–126 (1968).
51. Skirvin, D. J. & De Courcy Williams, M. Differential effects of plant species on a mite pest (Tetranychus urticae) and its predator (Phytoseiulus persimilis): Implications for biological control. *Exp. Appl. Acarol.* **23,** 497–512 (1999).
52. Jacobson, R. J., Croft, P. & Fenlon, J. Response to fenbutatin oxide in populations of Tetranychus urticae Koch (Acari: Tetranychidae) in UK protected crops. *Crop Prot.* **18,** 47–52 (1999).
53. Maniania, N. K., Bugeme, D. M., Wekesa, V. W., Delalibera, I. & Knapp, M. Role of entomopathogenic fungi in the control of Tetranychus evansi and Tetranychus urticae (Acari: Tetranychidae), pests of horticultural crops. *Dis. Mites Ticks* 259–274 (2009). doi:10.1007/978-1-4020-9695-2_21
54. Herron, G., Gough, N., Welliiam, T., Rophail, J. & Parker, R. Relationship Between Insecticide-Acaricide Resistance and Field Control in Tetranychus urticae (Acari : Tetranychidae) Infesting Roses. (1995).
55. McCrum-Gardner, E. Which is the correct statistical test to use? *Br. J. Oral Maxillofac. Surg.* **46,** 38–41 (2008).